The University of Nottingham

DATING KEROSENE RELEASES

KENNETH SCALLY, MSc.

Thesis submitted to the University of Nottingham
For the degree of Doctor of Philosophy

JULY 2013

This thesis is dedicated to

My parents, Esme and Pat Scally,

Pauline and our daughter Emma

"to accomplish great things we must not only act, but also dream, not only plan, but also believe" (Anatole France)

"in the midst of winter I finally learned that there was in me an invincible summer" (Albert Camus)

Pauline died 8th November 2012
(38 years of age)

"Life isn't about how to survive the storm, but how to dance in the rain"

Abstract

Kerosene is a common fuel for domestic heating systems. Dating petroleum spill contamination is of considerable international interest. An accurate determination of the age of spills is needed to inform the process of assigning legal and financial responsibility. The pollution of soils and groundwater by kerosene spills is of major concern to householders and their insurers as well as regulators. Released kerosene may persist in the soil as a source of hazardous hydrocarbons for a long time, but not as long as diesel, because of the low solubility and the moderate to low volatility of kerosene constituents. Generally, hydrocarbons in kerosene biodegrade significantly under aerobic conditions provided that sufficient amounts of essential nutrients are present. Extractable petroleum hydrocarbon (EPH) analyses by Jones Environmental Laboratories Ltd of soil polluted following kerosene spills were used to develop an empirical model which considered biotic and abiotic factors found at spill sites to determine the time since the kerosene spill.

Acknowledgements

The writer wishes to express sincere gratitude to Professor Paul Nathanail whose guidance and encouragement, generous commitment of time and advice at every stage of the research has been invaluable. Thanks also to the management and staff of Jones Environmental Forensics Limited and the Environmental Consultants for their co-operation and enthusiasm in collecting and processing data. Without their support and participation the study would not have been possible.

Many colleagues and friends helped; their generosity and practical help can never be repaid. To my family, who have nurtured me throughout the entire project, special thanks to my parents, and especially to Pauline and our daughter Emma.

Table of Contents

List of Figures

List of Tables

List of Equations

List of Appendices

Acronyms

Acronym	Meaning
$-CH_3$	Methyl Group
%	Per cent or Percentage
%NMC	Percentage Natural Moisture Content
%RSD	Percentage Relative Standard Deviation
+	Plus
>	Greater Than
±	Plus or Minus
π	Pi Bond
µl	Microlitre
µm	Micrometre
1e	Electron
A	Peak Asymmetry
ACT	Agricultural Central Trading Ltd.
Amu	Atomic Mass Units
ANOVA	Analysis of Variance
AQC	Analytical Quality Control
ASTDR	Agency for Toxic Substance and Disease Registry
BB	Baseline-to-baseline
BH	Borehole
BS	British Standards
BTEX	Benzene, Toluene, Ethylbenzene and Xylenes
C0	Parent Polycyclic Aromatic Hydrocarbon
C1	Methyl Substituted Alkylated Series
C2	Di-methyl Substituted Alkylated Series
C3	Trimethyl Substituted Alkylated Series
C4	Tetramethyl Substituted Alkylated Series
CRM	Certified Reference Material
CSV	Comma-Separated Value
CV	Cross Validation
DB-1	Durabond Non-polar Agilent Technologies Gas Chromatography Column
DCM	Dichloromethane
DECC	Department of Energy and Climate Change
DEFRA	Department for Environment, Food and Rural Affairs
DERV	Diesel Engine Road Vehicle
EA	Environment Agency
ECom	European Commission
EC	Equivalent Carbon
EPH	Extractable petroleum hydrocarbon
ESH	Engineering Statistics Handbook

Acronym	Meaning
EU	European Union
FID	Flame Ionisation Detection
Ft	Feet
Fuel Oil No. 1	Kerosene
Fuel Oil No. 2	Diesel
G	Gram
g/ml	Grams per millilitre
GC	Gas Chromatography
GC-1ht	Gas Chromatography high temperature
iC_{14}	Unknown isoprenoid
iC_{15} ($C_{15}H_{32}$)	Farnesane (iC_{15}: 2,6,10-trimethyldodecane)
iC_{16} ($C_{16}H_{34}$)	2,6,10-Trimethyl-tridecane
iC_{18} ($C_{18}H_{38}$)	Norpristane (iC_{18}: 2,6,10-trimethylpentadecane)
iC_{19} ($C_{19}H_{40}$)	Pristane (iC_{19}: 2,6,10,14-tetramethyl-pentadecane)
iC_{20} ($C_{20}H_{42}$)	Phytane (iC_{20}: 2,6,10,14-tetramethyl-hexadecane)
Id	Internal diameter
ISO17025	International Organization for Standardization General Requirements for the Competence of Testing and Calibration Laboratories
IUPAC	International Union of Pure and Applied Chemistry
JEFL	Jones Environmental Forensics Limited
Jet A	Aviation Fuel
L	Litre
m	Meter
M	Molecule
m	Mean
m/z	Mass to Charge Ratio
M^+	Parent or Molecular Ion
MADEP	Massachusetts Department of Environmental Protection
mg/kg	Milligrams per Kilogram
mg/l	Milligrams per Litre
MID	Multiple Ion Detection
Min	Minimum
ml/min	Millilitres per Minute
MS	Mass Spectrometry
MSD	Mass Selective Detector
MSE	Mean Squared Error
MSEr	The mean of squared error of the residuals
MW	Monitoring Well
n-alkane	Normal alkane
nC_1 (CH_4)	n-Methane
nC_8 (C_8H_{18})	n-Octane
nC_9 (C_9H_{20})	n-Nonane

Acronym	Meaning
nC_{10} ($C_{10}H_{22}$)	n-Decane
nC_{11} ($C_{11}H_{24}$)	n-Undecane
nC_{12} ($nC_{12}H_{26}$)	n-Dodecane
nC_{13} ($C_{13}H_{28}$)	n-Tridecane
nC_{13} ($nC_{13}H_{28}$)	n-Tridecane
nC_{14} ($C_{14}H_{30}$)	n-Tetradecane
nC_{14} ($nC_{14}H_{30}$)	n-Tetradecane
nC_{15} ($C_{15}H_{32}$)	n-Pentadecane
nC_{16} ($C_{16}H_{34}$)	n-Hexadecane
nC_{17} ($C_{17}H_{36}$)	n-Heptadecane
nC_{17} / Pr	Ratio of Heptadecane to Pristane
nC_{18} ($C_{18}H_{38}$)	n-Octadecane
nC_{18} / Py	Ratio of Octadecane to Phytane
nC_{20} ($C_{20}H_{42}$)	n-Isosane
nC_{22} ($C_{22}H_{46}$)	n-Docosane
nC_{24} ($C_{24}H_{50}$)	n-Tetracosane
nC_{26} ($C_{26}H_{54}$)	n-Hexacosane
nC_{28} ($C_{28}H_{58}$)	n-Octacosane
nC_{35} ($C_{35}H_{62}$)	n-Triacontane
nC_{40} ($C_{40}H_{82}$)	n-Tetracontane
nC_{60} ($C_{60}H_{122}$)	n-Hexacontane
NH_4^+	Ammonium
NIEA	Northern Ireland Environment Agency
NIST	National Institute of Standards and Technology
NO_3^-	Nitrate
NSO	Nitrogen, Sulphur and Oxygen
O_2	Oxygen
°C	Degrees centigrade
PAH	Polycyclic Aromatic Hydrocarbons
PFTBA	Perfluorotributylamine
pH	Potential Hydrogen
PM	Preparation Method
PO_4^{3-}	Phosphate
Ppm	Parts per million
Pr	Pristane
PT	Proficiency Testing
Py	Phytane
R^2	Coefficient of determination
RT	Retention Time
RTS	Retention Time Standard
S	Total Standard Deviation
SARA	Saturates, Aromatics, Resin and Asphaltenes

Acronym	Meaning
SCLF	Sulphur Content of Liquid Fuels
Sd	Standard Deviation
SEPA	Scottish Environment Protection Agency
SI	Statutory Instruments
SIM	Selective Ion Monitoring
SSC	System Suitability Chart
T	Time in years
TIC	Total Ion Chromatogram
TM	Test Method
TPH-7RPM	Florida total petroleum hydrocarbon standards mixture
TPHCWG	Total Petroleum Hydrocarbon Criteria Working Group
TRPH	Total Recoverable Petroleum Hydrocarbon
TUOA	The University of Arizona
UCM	Unresolved Complex Material or Unresolved Complex Mixture
UK	United Kingdom
UKAS	United Kingdom Accreditation Service
UNL	University of Nebraska-Lincoln
USEPA	United States Environmental Protection Agency

1.0 Chapter 1: Introduction

This opening chapter of the thesis comprises two sections. The first section introduces the study by presenting the primary research concern and research questions, the context within which the study is undertaken, the purpose of the research and the industrial motivations driving it. Following this, the second section provides a brief outline of each chapter of the thesis.

1.1 Background of the Research

Liquid petroleum (crude oil and the products refined from it) plays a pervasive role in modern society. Around the world today, legal proceedings surrounding instances of environmental contaminations are commonplace. Various petroleum products are involved in such cases, and include light distillate fuels like automotive gasoline, mid-range distillate fuels, such as jet fuels / kerosenes, fuel oil / diesel fuels and heavier marine diesel fuels, lubrication oils and residual petroleum products (Wade, 2001). Kerosene (Fuel oil No. 1) is one of the most common types of domestic home heating fuel oil spills. In 2008 the United Kingdom imported 528 thousand tonnes of kerosene for use as a heating oil and 7,961 thousand tonnes for aviation / turbine fuel (Department of Energy and Climate Change, 2008). In the article in the iPM BBC, Tracey (2008) estimated that 1.5 million UK households use domestic heating oil. Accidental oil spills or leakages from home heating oil storage tanks or pipelines may lead to contamination of soil, groundwater and eventually become a threat to drinking water. A significant proportion of the lower molecular weight compounds present in home heating oil are potentially toxic or mutagenic and require remedial action to restrict environmental damage following such a spill (Gouda, Sanaa, Chekroud et al., 2007; Greer, Fortin, Roy, et al., 2003). Such oil spills pose other problems for the homeowners as they may need to be relocated during remediation, especially if the cleanup of the kerosene contamination occurs under the dwelling. The structural disturbance on the property resulting from contractors' works (digging up floors) may result in significant costs which may exceed €100,000 (Nagel, 2010). Commonly, these costs are borne by the homeowner's insurance company, although the insurance company may try to obtain a contribution from the previous home owner's insurance company. For this reason, insurance companies retain law firms who often request information on the age of these releases (Oudijk, 2009). Due to the high costs, many of these residential cases end in litigation. Consequently, a legally defensible method to age these releases is needed.

Environmental forensic investigation chemistry is a scientific methodology developed for identifying petroleum related and other potentially hazardous environmental contaminants' and for determining their sources and time of release (Kaplan, Galperin, Lu et al., 1997). A common objective of many forensic investigations is to determine the timing of an oil spill. Particularly in cases of property ownership transfer, the timing of a kerosene spill can be a primary factor for fair allocation of the investigation and remediation costs. As such, forensic investigators often need to determine, or estimate, when a release to the subsurface occurred (Davidson and Creek, 2000). Forensic techniques available for dating and source identification of petroleum hydrocarbons include hydrocarbon pattern recognition, analysis of oxygenates, dyes, stable isotopes, weathering patterns, biomarkers and degradation models (Morrison, 2000). The most common technique now in use for the age determination of diesel is the Christensen and Larsen (1993) method. Their method however focuses on diesel and not kerosene. The purpose of this study is to develop and evaluate an alternative, semi-quantitative method for estimating the age of heating oil releases (kerosene spills).

1.2 Industry Goal

From an industry perspective this research work is expected to contribute to an enhanced understanding of the criteria associated with ageing the release of kerosene in home heating oil spills. Ageing spills of hydrocarbon products is a complex process. Empirical equations based on pristane and phytane contents of some 12 sites in Denmark have been widely used for diesel spills for the past two decades. It is anticipated that this research will develop and validate a model to predict the age of kerosene spills in typical UK and Irish environmental conditions. The model will be based on normal alkane / isoprenoid ratios extracted from several chromatograms. This industry objective is based on the premise that such a model will improve the capability of accurate allocation of responsibility to insurance companies. The relevancy of the use of the model is to allow insurance companies to apportion responsibility to the potentially responsible parties (PRPs). The model is only applicable to domestic kerosene oil spills.

1.3 Research Aim and Objectives

The primary aim of this thesis is to develop a protocol for chemical fingerprinting of spilled kerosene (domestic home heating oil) in order to estimate the age of release. At the outset of this research a number of specific research aims and objectives were formulated.

- To evaluate the preferential depletion of the n-alkanes dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil, by conducting a series of artificial weathering experiments (Chapter 4).

- To develop an analytical method for the rapid extraction of isoprenoids and n-alkanes content from chromatographic data (Chapter 5).

- To develop a database of chromatograms and associated metadata obtained from the analysis of kerosene spill samples (soil and water) received for analysis from 2009 to 2011 at Jones Environmental Forensics Limited. These samples have been submitted for analysis by environmental consultants working on domestic home heating oil releases in Ireland and the United Kingdom (Chapter 5).

- To analyse the database of isoprenoid and n-alkane data of kerosene spill samples (soil). To evaluate the environmental factors (weathering and biodegradation) affecting isoprenoids and n-alkane ratios in post-spill kerosene in soil in order to accurately age a spill event (Chapter 6).

- To develop an equation equivalent to the Christensen and Larsen (1993), Kaplan, Galperin, Alimi et al. (1996b) equation used for middle distillate, but based on forensic markers of isoprenoids and n-alkanes present in kerosene, which is applicable to kerosene spills (Chapter 6).

- To elicit criteria constraining the conditions within which the kerosene spill ageing equation can be relied upon (Chapter 6).

1.4 Structure of the Thesis

A diagrammatic overview of the thesis research presented in the eight chapters is illustrated in Figure 1.1. The thesis is structured into the following chapters introduction, literature review, methodology, analysis of kerosene weathering study, results, discussion and conclusion. A description of each chapter follows:

- **Chapter One – Introduction.** This chapter introduces the background to the research by setting out the industry goals and the research key aims and objectives. It further provides a layout and a brief summary of each of the chapters within the thesis.

- **Chapter Two – Literature Review.** This chapter contains an overall background discussion introducing the concepts and technology that form the core of this dissertation. This chapter provides a literature review and a knowledge base that can be used to enhance the understanding of the research results presented, as well as in some cases to provide additional details in related areas that have not been the focus of this research. The literature review discusses the fingerprinting of petroleum contamination through an understanding of the chemistry and composition of petroleum hydrocarbons. The weathering effects following a release with a focus on home heating oil (kerosene) is discussed. Finally, this chapter presents a review of the limitations of the use of the Christensen and Larsen method for the age estimation of diesel, in the context of developing a model for the attribution of liability and determination of age of kerosene release.

- **Chapter Three – Methodology.** The methodology employed to meet the research aims and objectives is described in this chapter. The analytical methods used to evaluate the weathering experiment and the analytical profile of kerosene is discussed.

- **Chapter Four – Analysis of Kerosene Weathering Study.** This chapter describes the laboratory experimental weathering study results. Initially the chapter provides an analytical review of the gas chromatographic profiling of kerosene using both flame ionisation detection and mass spectrometry. With the use of selective ion monitoring (SIM) utilizing gas chromatography coupled with mass selective detection the signature components in kerosene are identified. This chapter evaluates the preferential depletion of the n-alkanes dodecane (nC_{12}), tridecane (nC_{13}), tetradecane (nC_{14}) and pentadecane (nC_{15}) to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil. This was undertaken following a series of artificial weathering experiments on neat kerosene oil. The chapter concludes with a linear regression analysis of the level of evaporation versus the n-alkane to isoprenoid ratios found in kerosene.

- **Chapter Five – Results.** The results obtained in chapter four formed the bases for the development of a database of chromatograms and associated metadata. The database was populated with data obtained from the analysis of 6,100 sample chromatograms received for analysis from 2009 to 2011 at Jones Environmental Forensics Limited laboratory. The results of this data collection study along with a description of the analytical method developed for the rapid extraction of isoprenoid and n-alkane content from chromatographic data are presented. Environmental factors which may influence the model such as soil type, evaporation / volatilization, and biodegradation are discussed. Each of the models (Linear, exponential, power, polynomial and logarithmic) is statistically evaluated. Each of the models is validated using a cross-validation leave-one-out assessment. The models are compared using the mean squared error (MSE), percentage relative standard deviation (%RSD) and other descriptive statistical tools. Each model is then compared to the Christensen and Larsen (1993) and Hurst and Schmidt (2005) models.

- **Chapter Six – Discussion.** The discussion chapter chronicles in stages the design and development of the kerosene age estimation model. This chapter discusses the findings for each of the aims and objectives and finally presents the selected model. The chapter discusses the reasons for the eliminated data for the models when compared to the Hurst and Schmidt (2005) model.

- **Chapter Seven – Conclusion.** This chapter provides concluding remarks about the research aims and objectives and suggestions for future research that may arise from the work presented in this thesis.

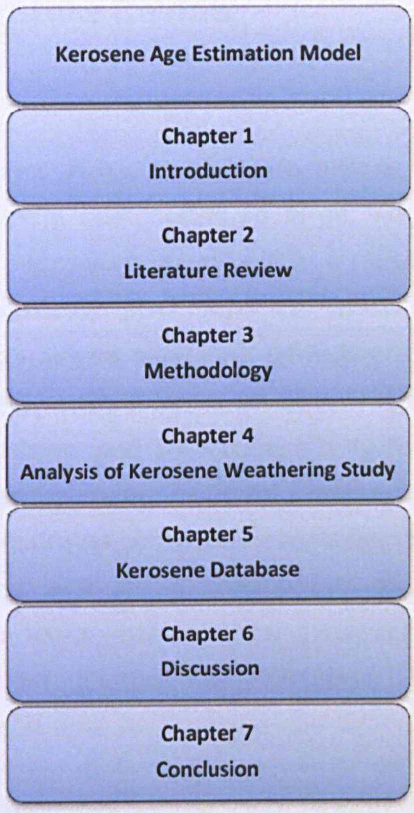


Figure 1.1 Structure of the Thesis

2.0 Chapter 2: Literature Review

Crude oils consist of complex mixtures of hydrocarbons and non-hydrocarbons that range from small, volatile compounds to large, non-volatile ones (Wang, Stout and Fingas, 2006b). Petroleum (crude oil) is a naturally occurring material of mainly plant origins, in particular phytoplankton, which is chemically converted under different geological conditions over long periods of time (Tissot and Welte, 1984). First formed in sedimentary beds, crude oil subsequently migrated to porous rocks such as sandstone and limestone. It is found at depths ranging from as little as 100ft to as much as 25,000ft (Peters, Walters and Moldowan, 2005). Crude oil primarily contains compounds comprising carbon and hydrogen. The composition can vary, and most constituent compounds can only be characterised by their elemental composition, however compounds occur in classes or groups of similar compounds (TPHCWG, 1998). It has been determined that crude oil contains heteroatom-containing nitrogen, sulphur and oxygen (NSO) organic compounds having more than 20,000 distinct elemental compositions ($C_cH_hN_nS_sO_o$) (Marshall and Podgers, 2004). In general, petroleum components are classified in bulk groups of **s**aturates, olefins, **a**romatics, **r**esin and **a**sphaltenes (this type of classification is also called SARA analysis) (Wang et al., 2006b). At the refinery, crude oil is separated by distillation into three main products: light (naphtha) distillate, middle distillate, and residual fractions (Alimi, Ertel, and Schug, 2003; Kaplan and Galperin, 1996a). The two fuel oil classes (kerosene and diesel) discussed in this thesis are refined from crude oil and may be categorised as distillate fuels.

2.1 Kerosene (Fuel Oil No.1)

Kerosene (Fuel Oil No.1), the main focus of this thesis, is a light distillate which consists primarily of hydrocarbons in the octane (nC_8) to hexadecane (nC_{16}) range (Figure 2.1). Kerosene, a flammable pale yellow or colourless oily liquid normally has a density of 0.80 g/ml at 20°C and is lighter but more viscous than water. It has a flash point of 38°C and a boiling range of 200°C to 290°C (Wang and Stout, 2007). Kerosene consists of 70% to 90% aliphatic hydrocarbons, 10% to 30% aromatics. For interpretation of kerosene by gas chromatography techniques there are two major groups of compounds which are used (ASTDR, 1995; Jones, 2003). These are aliphatic (saturates) and aromatic compounds.

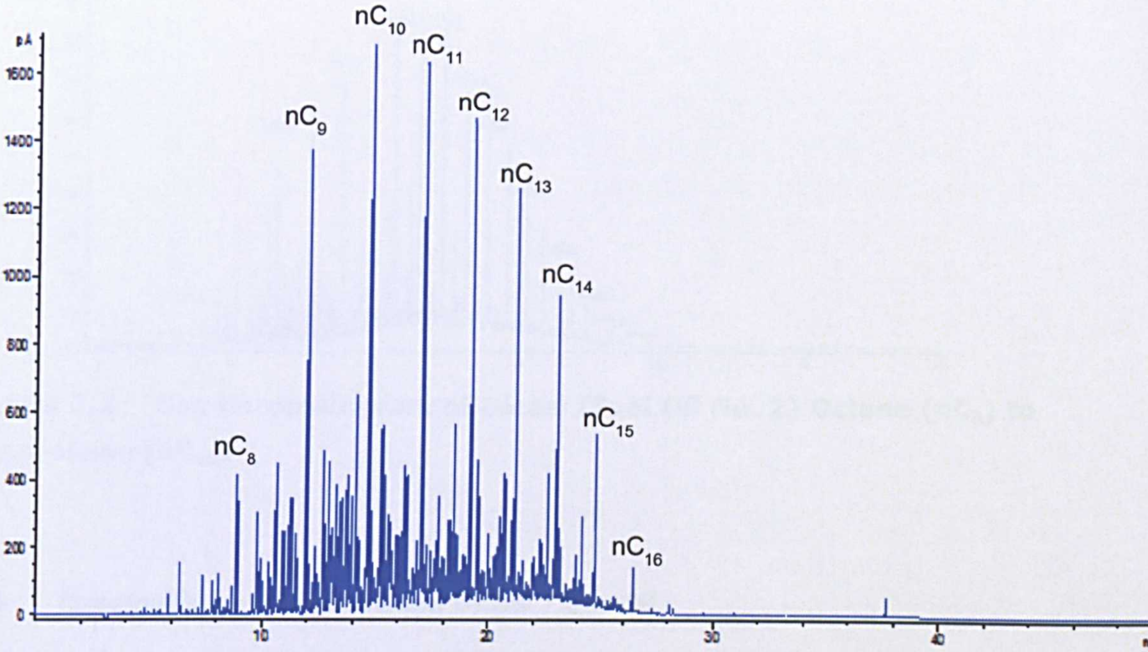


Figure 2.1 Gas Chromatogram of Kerosene (Fuel Oil No. 1) Octane (nC_8) to hexadecane (nC_{16})

2.2 Diesel (Fuel Oil No. 2)

Diesel fuels are classed as middle distillates. The major components of diesel are similar to crude oils, but include a higher aromatic fraction (10% to 40%) (Jones, 2003). The typical carbon range for transportation diesel is octane (nC_8) to octacosane (nC_{28}), however the vast majority of constituents are found in the dodecane (nC_{12}) to isosane (nC_{20}) range. Saturated hydrocarbons account for 60% to 90% of diesel (Jones, 2003). The majority are naphthenes which appear as an unresolved envelope when analysed by gas chromatography (GC). The recalcitrant isoprenoids pristane and phytane are also dominant and are used for forensic purposes (Jones, 2003). Figure 2.2 shows a typical chromatographic trace of diesel analysed on a gas chromatograph utilising flame ionization detection (FID).

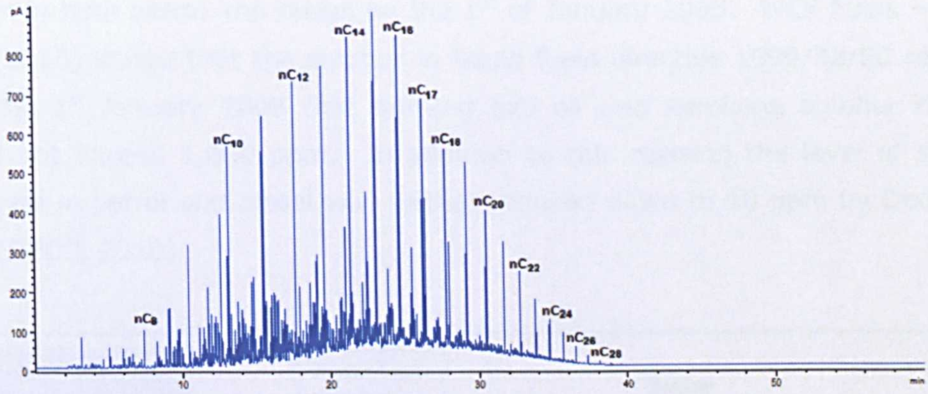


Figure 2.2 Gas Chromatogram of Diesel (Fuel Oil No. 2) Octane (nC_8) to Octacosane (nC_{28})

2.3 Composition of Kerosene and Diesel / Gas Oil

In 1993, the European Commission (EC) set up the 'Auto-Oil Programme' to work with European Union (EU) oil and motor industries to identify the most cost-effective means of improving air quality across Europe (Figure 2.3). These measures were designed to improve urban air quality across Europe by 2010 by reducing the level of sulphur in petroleum products. The European directive 98/70/EC, Fuel Specifications Directive was implemented on the 1st of January 2000 and required that:

- Petrol should contain a maximum of 150 ppm of sulphur.
- Diesel should contain a maximum of 350 ppm of sulphur (DECC, 2010).

This directive (98/70/EC) was transposed into UK law under the Motor Fuel (Composition and Contents) regulations 1999 (SI. 1999/3107). This statutory instrument (SI) covers the technical requirements for these fuels, including the chemical composition. The fuels also meet the requirements of the following British Standards (BS):

- 95 octane unleaded – BS EN 228:2004
- 97 octane unleaded – BS 7800:2004
- Diesel Engine Road Vehicle (DERV) – BS EN 590:2004 (DECC, 2010).

The Sulphur Content of Liquid Fuels (SCLF) directive (1999/32/EC as amended) came into force on the 27th of June 2000 for heavy fuel oil (Marine fuel) requiring the sulphur content to be set at a maximum of 0.1% (DEFRA, 2010). Under the "Auto-Oil 2 Program" the 98/70/EC directive was revised in 2003 (Directive 2003/17/EC) requiring that the limits would reduce to a maximum of 50 ppm of sulphur in both petrol and diesel by the 1st of January 2005. WCF Fuels – North West (2010) stated that the sulphur in liquid fuels directive 1999/32/EC requires from the 1st January 2008 that burning gas oil and kerosene sulphur content should not exceed 1,000 ppm. In addition to this revision the level of sulphur contained in petrol and diesel was further reduced down to 10 ppm by December 2009 (DECC, 2010).

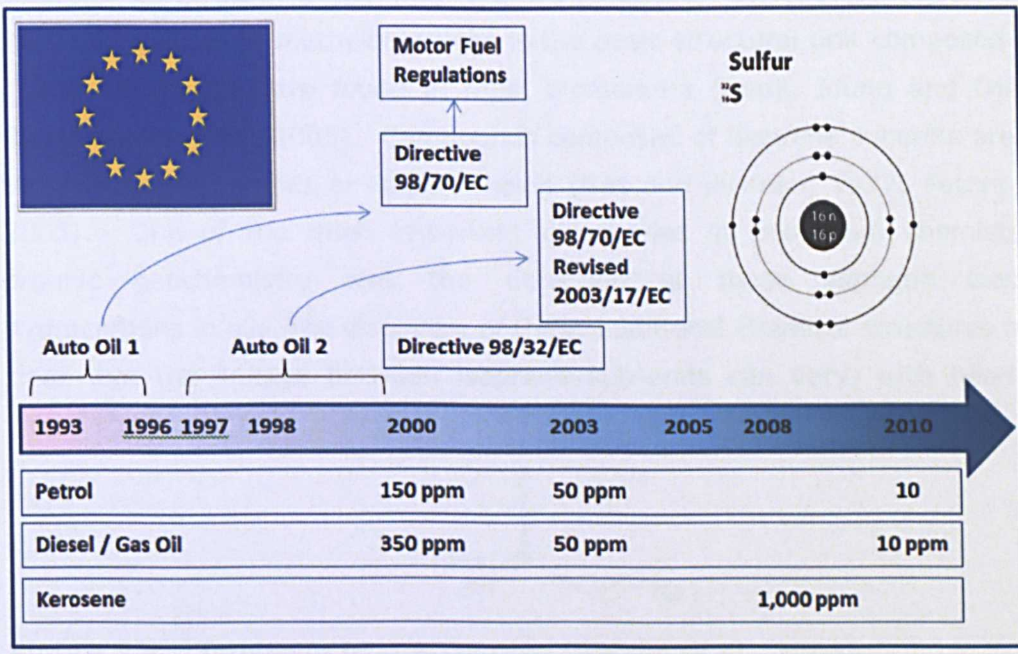


Figure 2.3 Sulphur Directives and Levels in Petrol, Diesel / Gasoil and Kerosene (DECC, 2010)

2.4 Distribution of Aliphatic Hydrocarbons in Fuel Oils

Aliphatics, sometimes also referred to as paraffins or alkanes are one of the major constituents of crude oil and are found in refined petroleum products such as gasoline, kerosene, diesel and heating fuels. The alkanes are not found in refined lubricating oils, except as a result of diesel dilution in used engine oil. Saturates are a group of hydrocarbons composed of only carbon and hydrogen with no double carbon–carbon bond. They are the predominant hydrocarbon classes that comprise crude oil (Peters et al., 2005). Saturates include straight chain and branched compounds ranging from methane (nC_1) to over hexacontane (nC_{60}). They are readily degradable by bacteria and compounds up to isosane (nC_{20}) are affected by weathering. The patterns observed in chromatograms of these alkanes are used to indicate the boiling range and to identity refined products.

2.4.1 Branched Aliphatics and Isoprenoids

Branched saturates are generally based upon the isoprene structure (Figure 2.4) and include the recalcitrant compounds called isoprenoids which are almost resistant to biodegradation. These hydrocarbons include some of the potential diagnostic signatures for the assessment and correlation between spilled oil and suspected source oils. Terpenoids are grouped according to the number of isoprene units from which they are biogenetically derived (Wang and Stout, 2007). Isoprene or methylbutadiene is the basic structural unit composed of five carbon atoms that are found in most biomarkers (Osuji, Idung and Ojinnaka, 2006; Peters et al., 2005). Compounds composed of isoprene subunits are called terpenoids, isoprenoids or isopentenoids (Nes and McKean, 1977; Peters et al., 2005). One of the most important discoveries in petroleum chemistry and organic geochemistry was the detection of these aliphatic isoprenoid hydrocarbons in oil. The discovery of these additional chemical structures made it clear that the linkage between isoprene sub-units can vary, with head-to-tail (regular) linkage and other (irregular) linkages occurring.

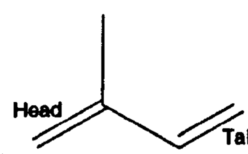
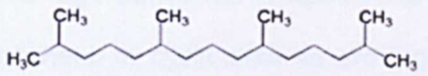


Figure 2.4 Isoprene (C_5) Structure (Peters et al., 2005)

Five of the most abundant and important oil isoprenoid compounds are:

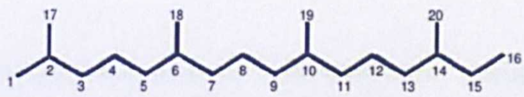
1. Farnesane (iC_{15}: 2,6,10-trimethyldodecane) which is an example of a regular acyclic isoprenoid consisting of three head to tail linked isoprene units;
2. 2,6,10-Trimethyl-tridecane (iC_{16});
3. Norpristane (iC_{18}: 2,6,10-trimethylpentadecane);
4. Pristane (Pr) (iC_{19}: 2,6,10,14-tetramethyl-pentadecane) is classified as a diterpane, although it contains one less methyl group ($-CH_2-$) than phytane; and
5. Phytane (Py) (iC_{20}: 2,6,10,14-tetramethyl-hexadecane) (Figure 2.5).

These compounds can be considered degraded diterpanes derived from the isosane (nC_{20}) parent by consecutive loss of methylene groups (Peters et al., 2005).



2,6,10,14-Tetramethylpentadecane

(Pristane)



2,6,10,14-Tetramethylhexadecane

(Phytane)

Figure 2.5 Pristane and Phytane (Peters et al., 2005)

The gas chromatogram of oil generated from a Forties blend crude oil supplied by SGS and analysed on a gas chromatogram by Jones Environmental Forensics Ltd. (Figure 2.6) illustrates these isoprenoids. The regular isoprenoid hydrocarbons (labelled in the insert as i-Carbon number)) are the most abundant resolved C_{10+} compounds after the normal alkanes (labelled by their carbon number). The minor, unlabelled peaks eluting between the n-alkanes are mostly other isoalkanes, monocyclic alkanes, and alkylated aromatic hydrocarbons. The isoprenoids pristane and phytane are identified as iC_{19} and iC_{20} respectively (Peters et al., 2005). The ratio between heptadecane (nC_{17}) and pristane can be used to determine the degree of degradation or age of the product in diesel and gas oils (Christensen and Larsen, 1993).

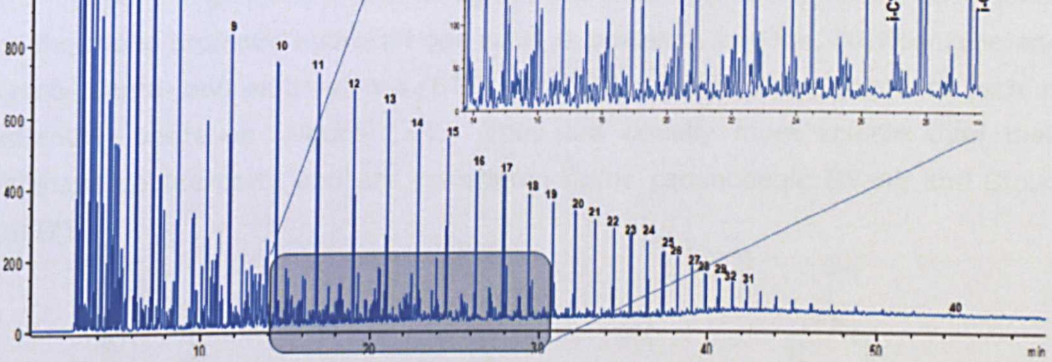


Figure 2.6 Isoprenoids Present in Forties Blend Crude Oil

2.4.2 Cycloalkanes (Naphthene)

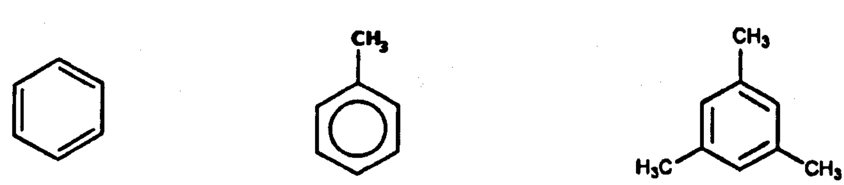
Naphthene or cyclic alkanes are highly complex compounds from the major group of constituents within a crude oil. They consist of many thousands of individual compounds which do not resolve on a chromatography column and are detected as an unresolved envelope referred to as a "UCM" or unresolved complex material (Kaplan, Lu, Alimi et al., 2001). The shape and extension of this UCM is used to assess the product type and molecular weight as well as the degree of biodegradation (Killops and Al-Juboori, 1990). Cycloalkanes are fairly resistant to aerobic biodegradation (Peters et al., 2005).

2.4.3 Alkenes

Alkenes, commonly referred to as olefins, are partially unsaturated hydrocarbons characterised by one or multiple double carbon-carbon bonds. These compounds are rare in crude oil but may be present in some petroleum products, having been formed during the refining process (Wang et al., 2006b).

2.4.4 Distribution of Aromatic Hydrocarbons in Fuel Oils

Aromatic hydrocarbons contain one aromatic carbon ring (benzene) which can be substituted with alkyl groups. Benzene is a one ring aromatic compound stabilised by a delocalised π electron system. The delocalized π bonds are more stable than the isolated double bonds or the single carbon to carbon bond which makes these compounds more resistant to remediation. These compounds, mainly found in gasoline and other lighter petroleum products, include compounds of the mono aromatic hydrocarbons such as benzene, toluene, ethylbenzene and ortho-, meta- and para-xylenes (BTEX) and other substituted compounds such as trimethyl benzenes (Figure 2.7). They are usually more soluble than their aliphatic counterparts and are considered to be carcinogenic (Wang and Stout, 2007).

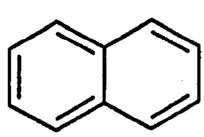


Benzene Toluene 1,3,5-Trimethylbenzene

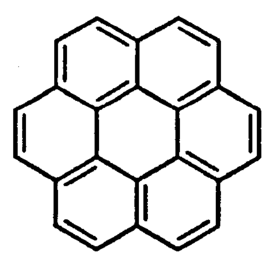
Figure 2.7 Benzene and 1,3,5-Trimethylbenzene (Peters et al., 2005)

2.4.5 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) contain two or more aromatic rings and are ubiquitous in the environment. They are formed by a variety of natural and anthropogenic processes, including diagenesis of organic matter, atmospheric soot residue from burning petroleum, wood, coal and petroleum releases. In aquatic environments, sediments are the primary location for these hydrophobic compounds (Costa, White and Ruspantini, 2004). Most PAHs derive from petrogenic (petroleum and coal) or pyrogenic sources (Zemo, 2009). PAHs contain two or more fused aromatic rings and range from naphthalene (two ring) to coronene (six ring) (Figure 2.8). These compounds also have alkylated derivatives such as methyl naphthalene and their derivative, and these can be used in assessing source differences (Costa and Sauer, 2005). These include the alkylated parent PAH (C0) to tetra-methyl substituted alkylated series (C4) naphthalene, phenanthrene, dibenzothiophene, fluorine and chrysene homologous series (Wang et al., 2006b). At least seven of the United States Environmental Protection Agency (USEPA) target compound lists are considered to be carcinogenic with benzo(a)pyrene being the most toxic (Morrison, 2000; Wang et al., 2006b; Wang and Stout, 2007). These are typically found in kerosene, diesel / gas oil and heavy fuel oils (marine diesel) and are generally absent in lubrication oil (Jones, 2003).



Naphthalene

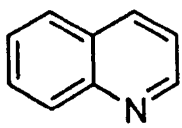


Coronene

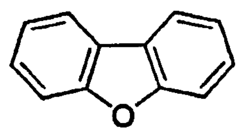
Figure 2.8 Polycyclic Aromatic Hydrocarbons – Naphthalene and Coronene (Peters et al., 2005)

2.4.6 Polar Compounds

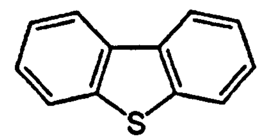
Polar compounds have distinct regions of positive and negative charge, as a result of bonding with atoms such as nitrogen, oxygen, or sulphur (Figure 2.9). In petroleum products, the smaller polar compounds are called resins (Wang et al., 2006b). Sulphur is typically the most abundant element in petroleum and may be present in several forms (Jones, 2003). Asphaltenes are a class of very large heteroatom-containing compounds (Wang and Stout, 2007). They are not dissolved in petroleum but are dispersed as colloids. Asphaltenes are generally classified, based on the solution properties of petroleum residues in various solvents as the oil constituents precipitated from oils and bitumen by natural processes or in the laboratory by addition of excess *n*-pentane or *n*-hexane (Wang and Stout, 2007).



Quinoline



Dibenzofuran



Dibenzothiophene

Figure 2.9 NSO Compounds (Peters et al., 2005)

2.5 Weathering Factors which Influence Kerosene

When petroleum products are accidently released to the environment, whether on water or land, they are immediately subject to a wide variety of changes in physical and chemical properties that in combination are termed weathering (Wang and Fingas, 2006a). Petroleum attenuation in soil is predominantly governed by:

1. Evaporation, occurring when petroleum is in contact with air and in soil, causing mainly lighter end petroleum constituents to volatilise;
2. Leaching, also known as dissolution or water washing, in which petroleum constituents dissolve into water; and
3. Biodegradation, the digestion of petroleum constituents by microbes (Oudijk, 2009).

The sequence of biodegradation according to Chapelle (2001) states that normal saturated hydrocarbons tend to be more readily available to convert to long-chained fatty acids for subsequent beta-oxidation than unsaturated or branch-chained hydrocarbons. This provides a source of considerable recalcitrance to degradation exhibited by some unsaturated and branch-chained aliphatic hydrocarbons.

Benzene, with its high solubility, tends to be the first aromatic compound to transition between phases (Kaplan et al., 1996). This depletion is normally followed by alkylbenzenes and alkylnaphthalenes. Alkylcyclohexanes are commonly more resistant than n-alkanes and may be found in the environment much later in the spill (Oudijk, 2009).

The rate of weathering of kerosene oils can be affected by the local environmental conditions during and after the spillage. Evaporation in the short term is the single most important and dominant weathering process (Wang and Fingas, 2006a). Weathering will cause changes in the chemical and physical properties of the spilled kerosene. Kaplan et. al. (1997) found that weathering could be divided into seven progressive stages:

1. Abundant n-alkanes;
2. Light-end n-alkanes removed;
3. Middle-range n-alkanes, benzene, toluene removed;
4. More than 90% of n-alkanes removed;
5. Alkylcyclohexanes and alkylbenzene removed;
6. Isoprenoids, methyl substituted alkylated (C1) naphthalenes, benzothiophene and alkylated benzothiophenes removed, dimethyl substituted alkylated (C2) naphthalenes selectively reduced; and
7. Phenanthrenes, dibenzothiophenes and other PAHs reduced.

Biodegradation including and beyond Stage 5 indicates substantial alteration and normally implies residence time greater than 20 years (Kaplan, 2003).

2.5.1 Abiotic and Biotic Weathering Index

Weathering can be categorised into two types, abiotic (physical) and biotic (microbial) weathering. The abiotic weathering is more predictable for middle distilates as the changes in n-alkanes are primarily affected. Wang and Fingas (2006a) suggested a weathering index for middle distillates which involved the sum of octane (nC_8), decane (nC_{10}), dodecane (nC_{12}) and tetradecane (nC_{14}) concentrations divided by the sum of docosane (nC_{22}), tetracosane (nC_{24}), hexacosane (nC_{26}) and octacosane (nC_{28}) concentrations (Equation 1).

$WI = \frac{(nC_8 + nC_{10} + nC_{12} + nC_{14})}{(nC_{22} + nC_{24} + nC_{26} + nC_{28})}$ Weathering Index (Wang and Fingas, 2006a)

Equation 1 Weathering Index for Gasoil / Diesel

Changes in chemical composition of petroleum fuels and the relative rate of their biodegradation are described in Kaplan and Galperin (1996) and are shown in Table 2.1. Changes in composition occurring below step 4 in the table imply aggressive weathering or environmental residence increasingly longer than 20 years.

Table 2.1 Change in Gasoline, Diesel Fuel and Bunker C Oil Composition During Biodegradation (Kaplan and Galperin, 1996b)

Fuel type	Level of biodegradation	Chemical composition
	1	Abundant n-alkanes
Gasoline	2	Light-end n-alkanes removed
	3	Middle-range n-alkanes, olefins, benzene, and toluene removed
	4	More than 90% of n-alkanes removed
	5	n-Alkanes quantitatively removed
		Alkylcyclohexanes and alkylbenzenes reduced
		Isoprenoids, C0-naphthalenes, and C0-phenanthrenes reduced
	6	C1-phenanthrenes begin to be removed
Diesel		Isoprenoids, C1-naphthalenes, benzothiophenes, and alkylbenzothiophenes removed
		C2-naphthalenes and C2-phenanthrenes selectively reduced
	7	All 2-ring PAHs degraded
		Dibenzothiophenes and other PAHs reduced
	8	Tricyclic terpanes enriched
Bunker C Fuel		Regular steranes relatively removed
		C31- to C35-homohopanes reduced
	9	Tricyclic terpanes, diasteranes, and aromatic steranes abundant
	10	Aromatic steranes and demethylated hopanes predominant

Increasing Level of Biodegradation

Atlas and Bartha (1992) concluded that whereas in one environment, spilled petroleum can persist almost indefinitely, under another set of conditions, the same hydrocarbons can be completely biodegraded within a few hours or days. Biodegradation depends on:

1. Presence of Microbiota with the metabolic capacity to degrade petroleum,
2. Recalcitrance of compounds in kerosene mixtures,
3. Growth and activity factors, such as temperature, nutrients, electron acceptors and pH, influencing the microbial population dynamics and
4. Bioavailability (de Jonge et al., 1997).

Microbiota with the potential to degrade hydrocarbons in soil and ground water include bacteria, fungi and yeasts although bacteria are normally the most significant. Dashti et al. (2008) found that the bacteria prefer the n-alkanes, while fungi prefer the oxidised byproducts. For biodegradation to occur, electron acceptors, such as oxygen (O_2) and nitrate (NO_3^-), and nutrients such as ammonium (NH_4^+) and phosphate (PO_4^{3-}) are needed. Aromatics can often be mineralised in the absence of O_2 under denitrifying, iron-reducing, methanogenic and / or sulphate-reducing conditions, while n-alkanes can mineralise under sulphate-reducing or denitrifying conditions (Bregnard et al., 1996)

The physical states, such as dissolved, or vapour phase, play an important role on the rate of weathering and biodegradation. Dissolved or vapour phases tend to be more susceptible to weathering processes. A larger the quantity of separate-phase may suppress or completely stop bacterial growth and reduce this mechanism of alteration. The degree of separate-phase spreading will also influence biodegradation. A thick pool separate-phase will biodegrade slower compared to a pool that has spread across a water table (Atlas and Bartha, 1992). A thinner pool or sheen will have more surface area in contact with ground water allowing for increased dissolution and volatilisation and hence more biological activity. The concentrations of hydrocarbons present in the dissolved or vapour phase can provide a strong influence on the petroleum degrading microbes.

Temperature near ground surface can deviate greatly while underground temperatures remain much more constant. An increase in temperature will lead to a general increase in the biological alteration processes. However at much colder temperatures, volatilisation of the lighter n-alkanes may decrease. Paved surfaces such as asphalt or concrete will have significant impacts on the temperature of near surface soil tending to retain heat. Grass-covered areas are more likely to cool quicker. Atlas and Bartha (1992) commented that with an increased temperature the viscosity of the petroleum product will decrease allowing for an increase in spreading providing additional surface area in contact with ground water and enhancing biological degradation processes.

Many of the compounds found in kerosene have low aqueous solubility with the aromatics being more soluble than aliphatics (Bobra, 1992). Dissolution of hydrocarbons into groundwater or soil will be influenced by the following factors:

1. The surface area of hydrocarbons in contact with water,
2. The ambient groundwater chemistry and in particular temperature, pH and redox,
3. The magnitude of precipitation and recharge, and
4. The groundwater migration rate.

Dissolution and biodegradation can be considered a coupled process as contact with water can stimulate biological activity (Oudijk, 2009).

The texture of the impacted soil can have an influence on the free movement of the released kerosene. The coarser-grained soil tends to allow for the movement of soil gas, percolating rain water and ground water which allows for the replenishment of oxygen, nutrients and microbes. Soil types such as a sand layer between clay will limit the replenishment of nutrients and oxygen, and any trapped kerosene may last for many years or decades (Oudijk, 2009).

The rate of alteration is predominantly influenced by the availability of oxygen and nutrients. Lack of oxygen can limit the aerobic microbes to degrade kerosene releases through natural attenuation. The factors which affect the availability of oxygen in soil (Atlas and Bartha, 1992; Oudijk, 2009) include the following.

1. Drainage: Water-logged soils oxygen diffusion can be slow and bacterial movement restricted.
2. Soil texture: More permeable coarse-grained soil allows for the replenishment of oxygen. The contact area between water and soil can also be increased enhancing dissolution.
3. Depth: Proximity to the ground surface allows for greater oxygen abundance, temperature variability and water percolation.

The soil and groundwater chemistry including pH or salinity may influence the biological rate of alteration of the released kerosene. Elevated salt concentration may limit microbes from consuming the kerosene. For the optimum microbial activity the pH is typically pH 8. The presence of some heavy metals in soils may also inhibit the microbial activity, as metals can be toxic to the microorganisms. The moisture can influence the rate of degradation, a lack of moisture normally exhibits a decreased biodegradation rate (Oudijk, 2009). The environmental factors impacting the weathering of kerosene are summarised in Table 2.2.

Table 2.2 Environmental Factors Impacting Weathering (Reproduced from Oudijk, 2009)

Environmental Conditions	Chemical Conditions
Soil permeability and porosity	n-alkane depletion
Water-table depth	n-alkane distribution
Soil cover	Aromatic content
Organic-matter content of soil	Unresolved complex mixture size
Groundwater salinity and pH Sulphide content of soil and groundwater Vegetation	Depletion of: Alkylcyclohexanes Alkylbenzenes

Environmental Conditions	Chemical Conditions
Dissolved oxygen content of ground water / oxidation-reduction potential (aerobic v. Anaerobic)	Thiophenes, Polyaromatic, branched alkanes
Moisture content of soil Temperature Indigenous microbial community Co-presence of other contaminants' Presence of electron acceptors Presence of emulsifying agents	Carbon range

The assessment of petroleum weathering through collection of soil or separate-phase samples and laboratory analysis with gas chromatograph (GC) equipped with flame ionisation (GC/FID) detection as described by Oudijk (2009) are categorised as very weak, weak, moderate, aggressive and very aggressive. These categories are based on specific environmental factors (Table 2.3).

Table 2.3 Site-specific Weathering Potential Regimes (Reproduced from Oudijk, 2009)

Regime	Example and Description
Very Aggressive	Water-logged soils (as per tank corrosion); areas prone to flooding; surficial or very shallow releases; stray electrical currents, heavily vegetated; nutrient-rich soils or groundwater, moderate pH (7-8); lack of soil cover; high recharge rates, and history of environmental pollution
Aggressive	High water table; highly-permeable and porous soils; lack of soil cover; shallow release; moderate pH (7-8); high oxygen content in soil and groundwater (>5 mg/l); high organic-matter content; stray electrical currents; high salinity content of groundwater; high soil sulphide content; high soil moisture; heavily vegetated, and urban environment (not pristine).
Moderate	Moderate water content in soil; moderate depth to groundwater; moderate permeability and porosity; moderate pH (5-9); moderate oxygen content in soil and groundwater (>2 mg/l); lack of stray electrical currents, and moderate vegetation.
Weak	Low moisture content in soils; water-logged soil (as per biodegradation); very high or very low pH; low oxygen content in soil and groundwater (<1 mg/l); deep water table; no stray electrical currents; low organic-matter content in soil; nonexistent or sparse vegetation, and pristine environment.
Very Weak	Extremely cold, hot or harsh environment; extremely high or extremely low pH; total lack of oxygen; pristine; no soil moisture, and sterilised environment.

2.6 Dating Diesel Fuels using the Christensen and Larsen Equation

The age dating of subsurface petroleum contamination is of considerable interest in Ireland, the United Kingdom and the United States. Because the legal and financial responsibility for hydrocarbon contamination is often decided based on the date of a fuel release, the importance of reliable tools for determining fuel release times is indisputable. One particular paper written by Christensen and Larsen in 1993 goes to address the subject of age dating of diesel in soil using the values of n-heptadecane (nC_{17}) to pristane (Pr). This paper has generated technical discussion within chemical literature journals. Litigation-driven expert witness testimony in courts in the United States has challenged the use of this model over the years (Brilis, 2002). There has been much discussion on its application which has highlighted some of the limitations in its use when applied to diesel spill sites in the United States (Alimi, 2002; Brilis, 2002; Kaplan, 2002; Stout, Uhler, and McCarthy, 2002a; Davis, Howe, and Nicholson, 2005). Several authors claim that the method is invalid (Stout, Uhler, and McCarthy, 2005). Oudijk (2010) has brought attention to two cases where the Christensen and Larsen (1993) model has been accepted by courts in the United States:

1. Champion Dyeing and Finishing Company Versus Centennial Insurance Company And North River Insurance Company, Superior Court of New Jersey Appellate Division, 2002; and
2. American Automobile Insurance Company and Maryland Casualty Company versus J.P. Noonan Transportation, Commonwealth of New Jersey, Superior Court Department, 2001.

In summary Christensen and Larsen measured a series of soil samples containing known releases of diesel from 12 service stations and oil terminal sites in northern Europe where a one-time only release of diesel (fuel oil No.2) reportedly occurred at a known time. They reviewed over 200 environmental reports of oil spills. Christensen and Larsen (1993) reported the soil temperatures at the 12 sites were approximately constant at 10°C with little or no remediation action taken prior to the time of the soil sampling. They were able to obtain a 22 year old diesel spill at Provestenen, Denmark where they performed a thorough investigation to examine the factors affecting the degradation process. The disparity in the rate of biodegradation among hydrocarbon classes is due to the isoprenoids pristane and phytane degrading more slowly than comparable n-alkanes Heptadecane (nC_{17}) and Octadecane (nC_{18}) (Stout et al., 2002a). The observed decrease in the Heptadecane (nC_{17}) / Pristane (Pr) ratio over approximately 20 years follows a well established path of preferential biodegradation of n-alkanes relative to isoalkanes (Kaplan and Galperin, 2008). This forms the basis for the method described by Christensen and Larsen, allowing for the use of the heptadecane (nC_{17}) / pristane ratio to date middle distillates.

The data from the Christensen and Larsen sites were found to have a linear correlation between the mean heptadecane (nC_{17}) / pristane ratio in the soil and the time in years since release (Figure 2.10) at a depth of 1m to 5m below the ground level and 1m above the water table (Stout et al., 2002a).

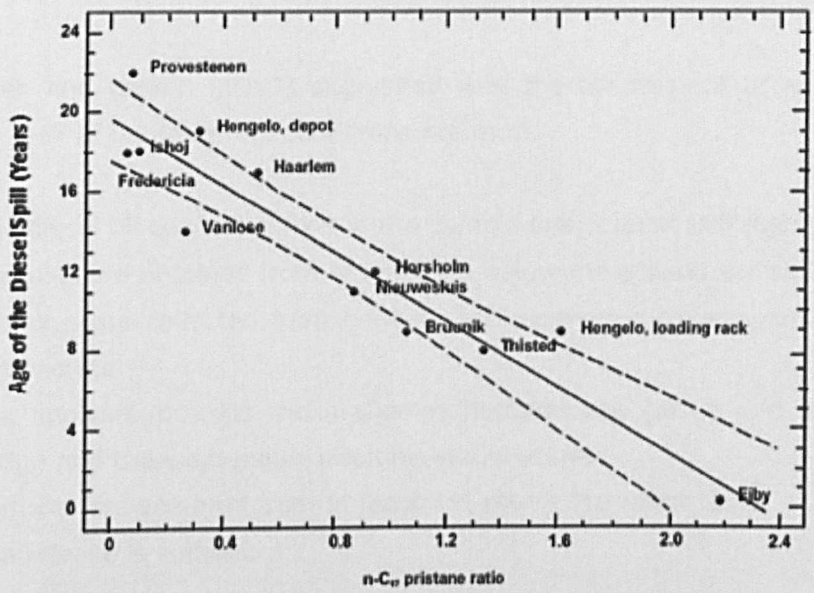


Figure 2.10 Christensen and Larsen Data Heptadecane (nC_{17}) / Pristane Ratio Versus Age in Years (Christensen and Larsen, 1993 Reproduced in Oudijk, 2009)

Christensen and Larsen (1993) reported age determined from heptadecane (nC_{17}) / pristane can be as accurate as ± 2 years at a 95% confidence level (Stout et al., 2002a; Kaplan and Galperin, 2008). As the biodegradation proceeds, the heptadecane (nC_{17}) / pristane ratio will decrease. Christensen and Larsen (1993) reported fresh diesel ratios of 2.0 to 2.2 dropping to 0.2 within soil over 20 years. Kaplan, Galperin, Alimi et al. (1996b) provided an equation to simplify the observations of the Christensen and Larsen age equation (Equation 2) (Figure 2.11). As a result, to determine the age of a release, the heptadecane (nC_{17}) /

pristane ratio is determined from the sample chromatogram and inserted into the formula to calculate the age within ± 2 years.

$$T = -8.4\left({}^{nC_{17}}/_{Pr}\right) + 19.8$$

Where T = the time in years since the diesel spill

Heptadecane (nC_{17}) / Pristane (Pr) = the ratio of the peak heights for Pristane (Pr) and heptadecane (nC_{17}) alkane obtained from the chromatogram

Equation 2 Kaplan Heptadecane (nC_{17}) / Pristane Equation based on Christensen and Larsen 1993 Study (Kaplan et al., 1996b)

Christensen and Larsen (1993) suggested that the assessment of age may be valid only if all of the following conditions are met.

1. The diesel oil concentrations in the sample are at least 100 mg/kg.
2. Samples are obtained from at least 1m below the ground surface.
3. Samples are collected from beneath an impervious cover such as asphalt or concrete.
4. The analysis includes the n-alkanes heptadecane (nC_{17}) and octadecane (nC_{18}) and the isoprenoids pristane and phytane.
5. Samples are acquired from at least 1m above the water table.
6. The release is sudden.

Concentrations of diesel in soil greater than 100 mg/kg provided Christensen and Larsen with a high degree of certainty that the oil-affected soil would not be affected by microbial degradation. Stout, Uhler, McCarthy et al. (2002b) suggested that a maximum concentration may also be warranted because soils that are completely saturated with petroleum may be toxic to microbes, lessening the rate of biodegradation. Samples collected from soil covered by asphalt or concrete would have a limited effect of leaching due to water washing because of the surface protection. This constraint would significantly reduce the effects of volatilisation. This precludes the use of these methods for determining the age of surface spills, such as overfills, piping failures or aboveground tank leaks. The effect of collecting a sample 1m above the water table is to minimise dissolution caused by contact with ground water and to minimise capillary fringe effects.

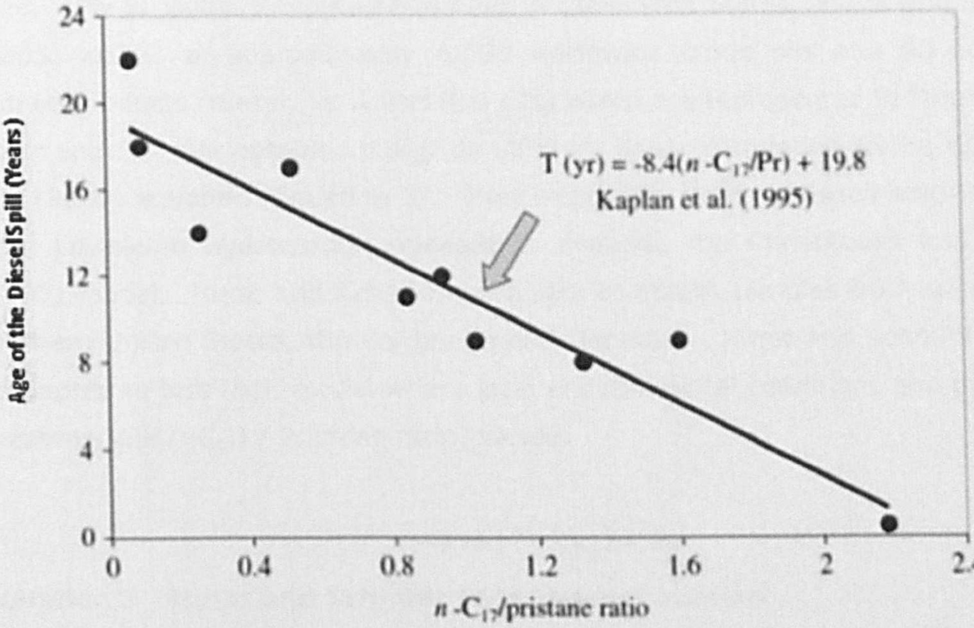


Figure 2.11 Regression Model for Age Dating Middle Distillates (Stout et al., 2002b)

There are a number of caveats that must be considered when utilising this dating method. The approach can be a useful tool but should be restricted to sites where a sudden, single release of fuel has occurred. A slow continuous fuel release or consecutive fuel releases into the same soil will invalidate the method (Christensen and Larsen, 1993; Kaplan and Galperin, 2008). In order to perform any environmental forensic study, it is always important to understand the soil conditions pertaining to the site. Dry conditions in soil or stagnant conditions in groundwater will suppress the rate of alkane attenuation, whereas rapid water flow will bring oxygen to the site and enhance biodegradation. Among other parameters affecting applicability of the dating approach are the site hydrology, soil temperature, moisture content, and availability of oxygen and nutrients (Atlas and Bartha, 1992; Kaplan et al., 1997). Understanding the site conditions is a prerequisite to understanding rates of weathering. Some parameters will favour fuel persistence and others will strongly promote the weathering of the spilled fuel. The Christensen and Larsen ratio can be used to derive an approximate residence time of diesel in the environment, recognising the limitations presented by the assumption that the original heptadecane (nC_{17}) / pristane ratio is 2.2. It is important to note that the study only looked at diesel fuels in northern Europe at the time and did not extend its applicability of the method to fuel from different countries which may have a broad range of starting heptadecane (nC_{17}) / Pristane ratio. This is partially applicable to fuels in the United States. Hurst and Schmidt (2005) looked at approximately 4,500 worldwide crude oils and 90 domestic refined products (diesel, jet A and fuel oils) which are represented in Figure 2.12. Hurst and Schmidt obtained a slightly different linear correlation to the Kaplan et al. (1996b) equation (Equation 3). They integrated both published and new data from petroleum hydrocarbon releases to evaluate the Christensen and Larsen (1993) model. Hurst and Schmidt were able to obtain samples from sites in the southern United States, the Caribbean and Denmark. Hurst and Schmidt (2005) attempted to test their model where local environmental conditions and the initial (heptadecane (nC_{17}) / Pristane ratio) varied.

$$T = -9.76 \left({}^{nC_{17}}/_{Pr} \right) + 20.7$$

Equation 3 Hurst and Schmidt (2005) Age Equation

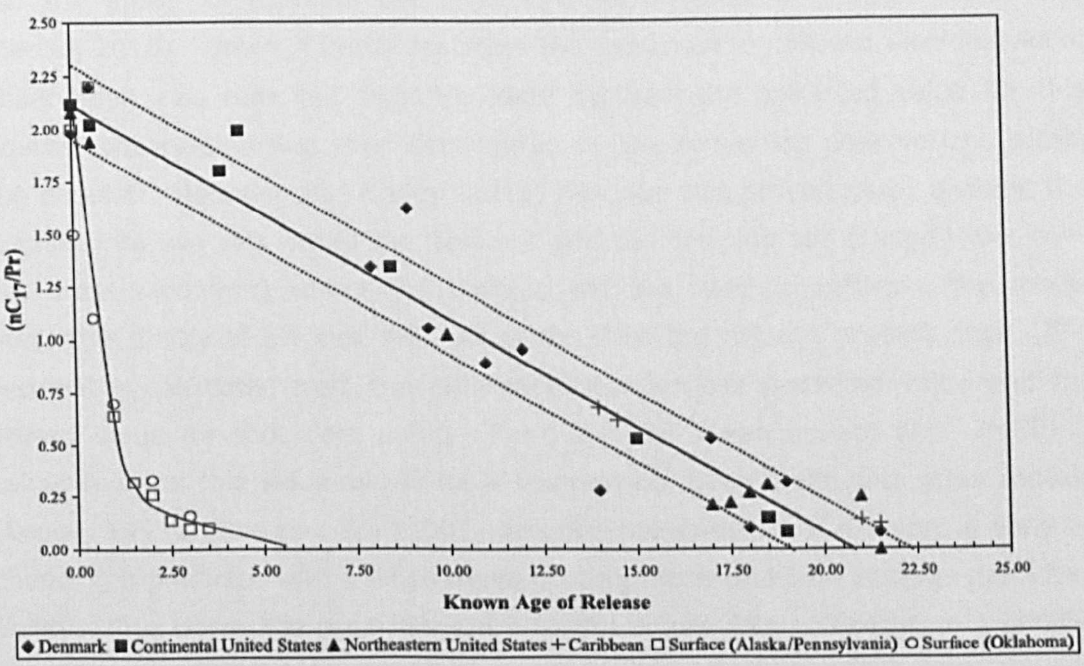


Figure 2.12 Middle Distillate Degradation Model (Hurst and Schmidt, 2005)

Hurst and Schmidt (2005) found an initial heptadecane (nC_{17}) / Pristane (Pr) ratio of 2.12. This ratio was based on a weighted mean of the data set of samples Hurst and Schmidt reviewed within their study. They reported that they were able to estimate the age of a release within ± 1.5 years under optimal conditions and ± 5 years under the worst case conditions (Hurst and Schmidt, 2005). Hurst and Schmidt reported that under aerobic environmental conditions that surface releases weathered rapidly with the heptadecane (nC_{17}) being depleted within about 6 years. They found that the model is not applicable to surface releases. Under anaerobic conditions the model Hurst and Schmidt produced ranged from ± 1.5 years to ± 5 years and concluded that in cases where the error was ± 5 years they were only able to conclude the release was older or younger than 10 years (Hurst and Schmidt, 2005).

2.7 Model Selection and Validation

Cross-validation is widely used as a model assessment and selection tool (Bergmeir and Benitez, 2012; Droge, 1999; Anderssen, Dyrstad, Westad et al., 2006). Cross-validation utilises all samples within a model for training and testing purposes (Gidskehaug, Anderssen and Alsberg, 2008). Cross-validation has the ability to evaluate the predictive performance of models (Borra and Ciaccio, 2010). Droge, (1999) describes the technique as follows: each individual observation was removed from the data set and the predicted value for this omitted observation was then determined by the remaining observations within the dataset. Maunder and Harley (2011) describe this procedure by dividing the dataset into two sets called the 'test set' and the 'training set' (called leave-one-out cross-validation) where the training set are used to estimate the model predictive ability of the test set. All of the data are left out at least once. The residual is calculated from the difference between the predicted value and the known value for that data point. From this the mean square error (MSE) is calculated and this value allows for a comparison to be made with other models (Droge, 1999). Rao and Wu (2005) describe the selection of a model is done by choosing a predictor with a small mean squared error and best average predictive ability. The closer the predictions are to the actual data will result in a smaller value for the MSE_r (TUOA, 2012).

3.0 Chapter 3 Methodology

The primary aim of this thesis described in Chapter 1 is to develop a protocol for chemical fingerprinting of spilled kerosene (domestic home heating oil) in order to estimate the age of release of this product at domestic properties. The model generated will be based on the distribution of normal alkane / isoprenoid ratios extracted from several chromatograms. The model's development is described within this Chapter. This industry-led objective is based on the premise that such a model will improve the capability of accurate allocation of responsibility to insurance companies for home heating oil spills. This closes a significant gap in the existing research.

This chapter describes and defends the methodology chosen for this research. It investigates the research questions in more depth, and discusses what methods are the most appropriate, given the aims and nature of the research. It describes the chosen set of methods of data collection and analysis; and the development of the kerosene estimated age of release model. The subsequent sections describe the methodology adopted for each of the objectives introduced in Chapter 1.

Five specific research aims and objectives described in Chapter 1 were initially formulated and this Chapter reviews the methodologies employed to meet each of these objectives.

3.1 Research Question 1 - Kerosene Profile and Weathering Experiment

As described in Chapter 2 Literature Review, the rate of weathering of kerosene oils can be affected by the local environmental conditions during and after the spillage. Wang and Fingas (2006a) described evaporation in the short term as the single most important and dominant weathering process. The impacts of weathering will cause changes in the chemical and physical properties of the spilled kerosene. The first research objective was to evaluate the preferential depletion of the n-alkanes dodecane (nC_{12}), tridecane (nC_{13}), tetradecane (nC_{14}) and pentadecane (nC_{15}) to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil, by conducting a series of artificial weathering experiments within the laboratory at Jones Environmental Forensics

Ltd. Kerosene exhibits an identifiable and often unique compositional profile as determined by gas chromatography (GC) utilising flame ionisation detection. The measurement of this profile is described in detail in Chapter 4. This profile was determined by the use of the method for the determination of extractable petroleum Hydrocarbons (EPH) (Appendix 1). Chapter 4 - *Analysis of Kerosene and Weathering Experiment* utilises the following methodology to achieve this objective.

3.2 Chromatography of Kerosene

The characterisation of the released petroleum oils is often referred to as profiling or fingerprinting (Pasadakis, Gidarakos, Kanellopoulou et al., 2008). This is an integrated analytical and data processing methodology that aims to reveal the affiliation of the contaminants' chromatographic profile to a group of chemically similar objects and to identify the pollution source(s). Kerosene exhibits an identifiable and often unique compositional profile as determined by gas chromatography (GC) utilising flame ionisation detection (Figure 3.1).

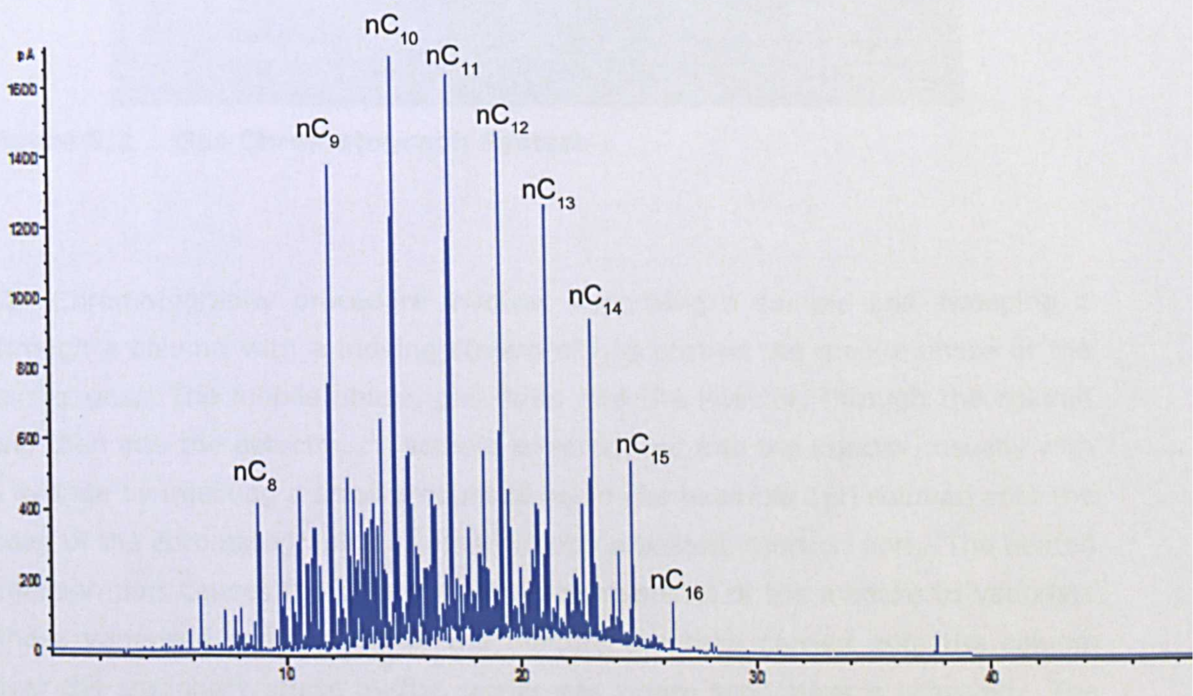


Figure 3.1 Kerosene Profile by GC/FID

3.3 Gas Chromatography Separation of the Components of Kerosene

The fingerprint in Figure 3.1 is obtained by a Gas Chromatography (GC) (Figure 3.2) system which is described as an instrumental method for the analytical separation and identification of chemical compound mixtures. This is achieved by separating the sample into individual components, it is then easier to identify and measure the amount of various sample components found.

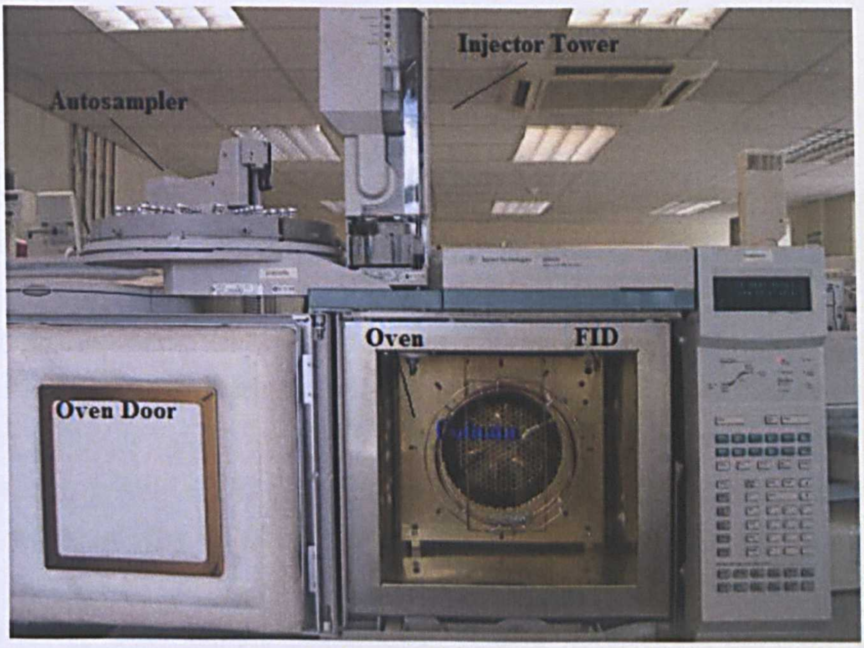


Figure 3.2 Gas Chromatograph System

Gas Chromatography procedure involves vaporising a sample and sweeping it through a column with a moving stream of gas termed the mobile phase or the carrier gas. The mobile phase, gas flows into the injector, through the column and then into the detector. A sample is introduced into the injector, usually with a syringe by injecting a small amount of liquid (for example 1μl) solution onto the head of the chromatographic column through a heated injection port. The heated injection port causes the volatile sample components of the mixture to vaporise. These vaporised components of the mixture are then carried onto the column over the stationary phase by the carrier gas where separation is achieved. The stationary phase in gas chromatography is a column which is a length of tube (Capillary Column) which can be up 30 meters in length coated with a high boiling point liquid (Figure 3.3). The components of the mixture transition through the column at a rate primarily determined by their physical properties and the temperature program of the GC oven.

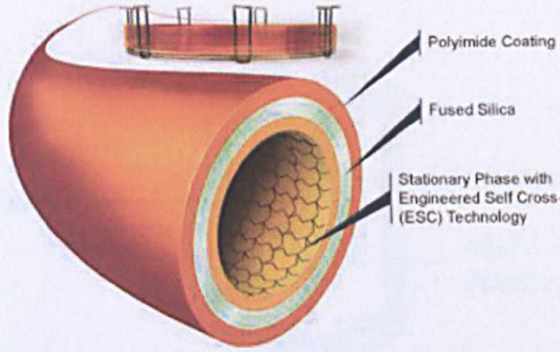


Figure 3.3 GC Column (Reproduced from Phenomenex, 2010)

The components leave the column and elute in order of volatility with the most volatile first (Jones, 2003). The components of the mixture are carried through the column by a stream of inert gas typically helium (mobile phase). The more volatile the component and the less interaction with the stationary phase the faster it travels through the column (Anon., 2010a; AnalChem Resources, 2010). Since each of the components of the mixture have different physical and chemical properties, some will be more slowed down by the stationary phase than others, therefore some of the components will move through the column at different speeds. This separation is achieved by a combination of factors such as boiling point, solubility and compound affinity as the component mixture partitions between the mobile phase and the stationary phase (Jones, 2003). As compounds have different rates at which they move through the column they exit the column at different times. As the components are separated, they elute from the column and enter a detector. The detector is creates an electronic signal whenever the presence of a component is detected. A flame ionisation detector (FID) is a typical detector used to fingerprint kerosene releases (Figure 3.4). A flame ionisation detector consists of hydrogen flame burning in air. As the components elute from the column it enters this flame and burns which produces ions. These ions can be detected by measuring the electrical conductivity of the flame between two plates. The concentration of the component is directly proportional to the electric current measured by the detector.

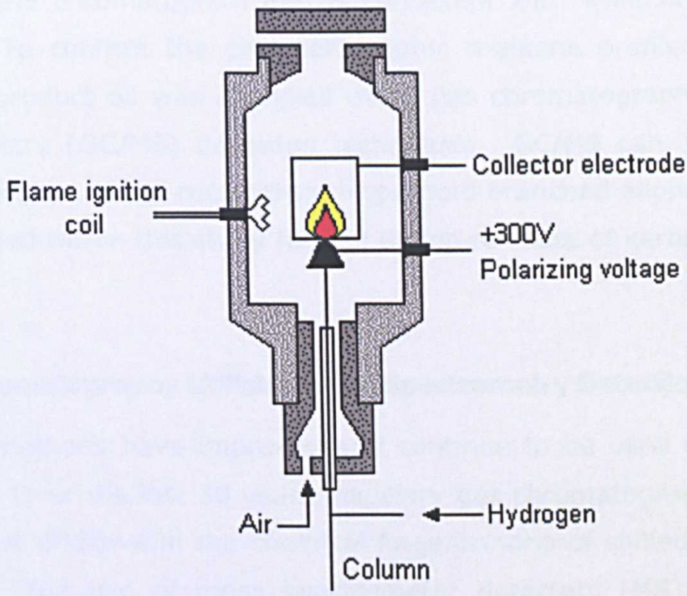


Figure 3.4 Flame Ionisation Detector (FID) (Reproduced from UNL, 2010)

3.4 Determination of the Profile Characteristics of Kerosene by GC/FID

Before beginning a separation, the flow rate of the gases and the temperature conditions of the oven on the gas chromatograph are set. The temperature of the inlet port is set to a temperature which ensures that the sample mixture is fully vaporised. A ChemService standards TPH-7RPM, *Florida total petroleum hydrocarbon standard solution mixture* mix of n-alkanes from octane (nC_8) to tetracontane (nC_{40}) and a Polycyclic Aromatic Hydrocarbon (PAH) standard mixture is injected onto the top of the column to determine the retention time of each of the individual n-alkane and PAH components within the mixtures. The more volatile components will elute first and the retention time can be recorded. The retention time is the time from when the injection is made (time zero) to when elution occurs at the detector and is referred to as the retention time (RT). The retention time is characteristic of individual compounds and identification can be achieved by comparison with known substances (Jones, 2003).

The unique profile resulting from the analysis of kerosene oil obtained by GC/FID represented illustrates an n-alkane distribution from octane (nC_8) to hexadecane (nC_{16}). This carbon range is based on the retention time profile determined by using the Florida n-alkane standard mixture as described above. The characteristic unresolved complex mixture (UCM) found in petroleum products is evident within the chromatogram and is consistent with what is expected from kerosene oil. To confirm the chromatographic n-alkane profile of kerosene a sample of the product oil was analysed using gas chromatography coupled with mass spectrometry (GC/MS) detection techniques. GC/MS can also be used to confirm the presence of the recalcitrant isoprenoid branched aliphatic compounds which will be used within this study for age dating releases of kerosene.

3.5 Gas Chromatography Utilising Mass Spectrometry Detection

Spectroscopic methods have improved and continue to be used in some oil spill investigations. Over the last 30 years, capillary gas chromatography (GC) clearly has proven most effective in the chemical fingerprinting of spilled oils (Wang and Stout, 2007). The use of mass spectrometer detectors (MS) has long been recognised as the most powerful detector for a gas chromatograph (GC) due to its high sensitivity, specificity and its capability to elucidate compound structures. Bench top quadrupole GC/MS systems are now commonplace in modern laboratories.

Mass Spectrometry is defined as the study of systems causing the formation of gaseous ions, with or without fragmentation, which are then characterised by their mass to charge ratios (m/z) and relative abundances (Anon., 2010b). The purpose of the mass spectrometer is to provide a fragmentogram which is a breakdown chromatogram of the structure of the molecule (M). Following separation by column chromatography as described the individual components elute from the GC column and enter the electron ionisation chamber of the mass spectrometer. Here the molecules are bombarded with a stream of high energy electrons (1e). These electrons have a high enough energy to knock an electron off the molecule as they enter the mass spectrometer. This stream collides with the molecules as they enter which produces a radical ion which has a positive charge and is given the symbol (M^+). This radical ion, M^+ is called the parent ion or molecular ion. The molecular ions are unstable, and break up into smaller pieces. These ions are then accelerated within an electric and magnetic field towards the detector. Ions of different mass enter the detector by varying the electric and magnetic field during the analysis instrument run. The amount of deflection within the magnetic field depends on the mass to charge ratio (m/z) of the ion. The majority of ions only have a single charge. Heavier ions may not be deflected sufficiently by the magnetic field to reach the detector and lighter ions will be deflected too much. During an analysis the magnetic field is gradually increased so that ions of successively greater mass enter the detector. The mass spectrum of the isoprenoid farnesane (iC_{15}: 2,6,10-trimethyldodecane) is represented in Figure 3.5. Each vertical line represents a different ion and its mass in relative atomic mass units (amu) is shown on the horizontal axis. The height of a peak represents the abundance of the ions. The peak at mass to charge (m/z) 212 represents the parent ion (M^+), which is the whole molecule which has lost one electron in the ionisation chamber. The peak at m/z 197, which is formed, is a result of m/z 212 losing 15 amu. This peak at m/z 197 is the parent ion losing one methyl ($-CH_3$), groups of relative molecular mass 15, which have broken off within the ionisation chamber. Other peaks within the fragmentogram are formed by more complex breakdowns and rearrangements within the mass spectrometer (RSC, 2010).

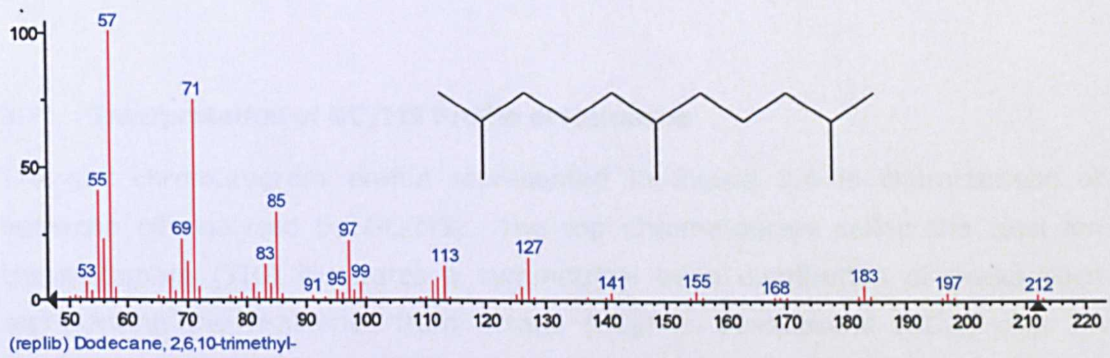


Figure 3.5 Farnesane (iC_{15} : 2,6,10-trimethyldodecane)

3.6 Determination of the Profile Characteristics of Kerosene by GC/MS

The conditions which have been used to determine the profile of kerosene within this chapter using a GC/MS system at Jones Environmental Laboratory are as follows.

- Analyses of kerosene was performed on an Agilent 7890 GC coupled with an Agilent 5975B inert mass selective detector (MSD).
- System control and data acquisitions were achieved with the Agilent MSD Chemstation chromatographic software (E.02.00.493).
- A Greyhound GC-1ht 15 m x 250 µm i.d. (0.10 µm film thickness) fused-silica capillary column was used.

The chromatographic conditions were as follows:

- Carrier gas, helium (1.2 ml/min),
- Injection mode, split mode, split ratio 1:1,

- Injector and detector temperature, 320°C and 300°C respectively,
- The temperature programme employed for the study is, 45°C hold for 4 minutes, then ramp at 7°C/min to 300°C,
- Prior to sample analysis, the GC/MS is tuned with perfluorotributylamine (PFTBA). The total run time was 40 minutes.

All solvents used were of the highest purity available. A sample of kerosene was prepared by adding approximately 50µl of the kerosene oil purchased from a commercially available source. ACT (Agricultural Central Trading Ltd., 90 The Broadway, Chesham, Buckinghamshire, HP5 1 EG), dissolved into 1.5mls of dichloromethane (DCM) in a 2ml clear crimp topped vial. This source was selected as it best represented and reflected the typical spilled product found within this study.

3.7 Interpretation of GC/MS Profile of Kerosene

The gas chromatogram profile represented in Figure 3.6 is characteristic of kerosene oil analysed by GC/MS. The top chromatogram called the total ion chromatogram (TIC) illustrates a symmetrical even distribution of peaks each representing the n-alkanes from octane (nC_8) to hexadecane (nC_{16}) over an unresolved complex mixture. The Agilent Chemstation software allows the GC/MS operator to select individual peaks in the TIC to view their ion fragmentogram. In this example, the n-alkane peak just before the ten minute retention time, decane (nC_{10}) was selected by moving the cursor over the peak and double clicking on it. This then reveals the bottom mass spectral fragmentogram which represents the ions collected by the mass spectrometer at this retention time (Figure 3.6).

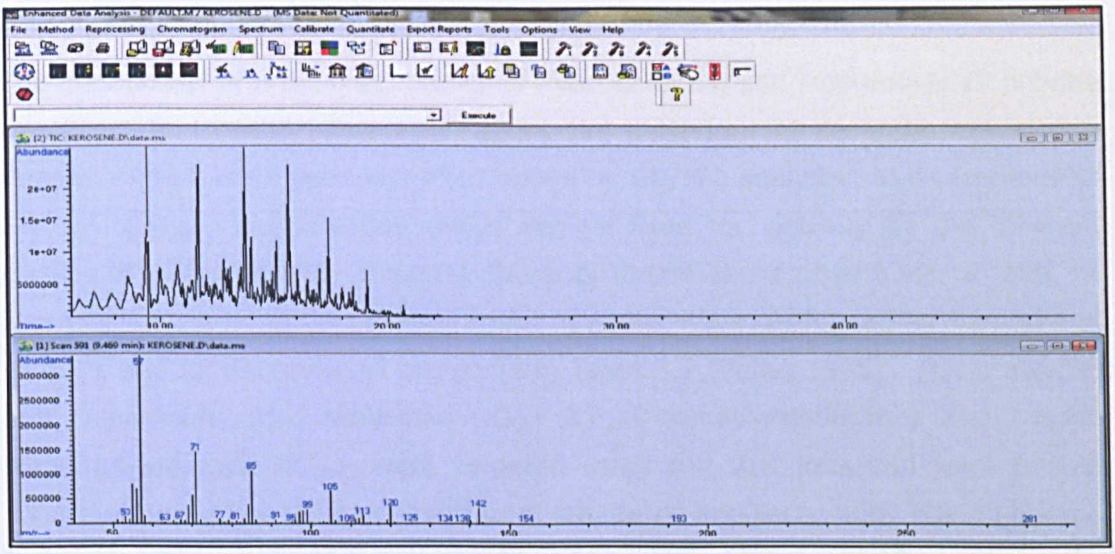


Figure 3.6 Kerosene Total Ion Chromatogram (Top) and Fragmentogram of Decane (Bottom)

3.8 Using Selective Ion Monitoring (SIM) in the Profiling of Kerosene

GC/MS systems can be operated in various modes, including the most widely used scan mode and selected ion monitoring (SIM) mode. Several different types of scan mode (data acquisitions) can be used in mass spectrometry, depending on the nature of the information sought. The data acquisition described earlier provides total ion chromatograms where each individual peak is representative of the mass to charge ratio of ions specific to the individual molecules eluting from the chromatography column into the mass spectrometer. This type of data acquisition is carried out in scan mode. If the maximum amount of information is desired full scan mode is typically used and the m/z data are collected over the entire mass range. Selected ion monitoring (SIM) is an alternative to full scan mode. SIM allows the operator to measure a small number of masses, typically the molecular ion and a number of characteristic masses. The SIM mode allows for the selection of the signature mass to charge ratio (m/z) ions which can be used to target compounds. In selected ion monitoring the mass spectrometer is set to scan over a very small mass range, typically one amu. By targeting only the ions with the specific m/z ratio, compounds with the selected mass can be detected and plotted (Philp, 2004).

3.9 SIM Profiling of n-Alkanes and Isoprenoids in Kerosene

The distribution of n-alkanes, branched hydrocarbons, and isoprenoids all provide significant and useful information about the behaviour of kerosene following a release. These have been identified above by GC/FID analysis. The technique of SIM or multiple ion detection (MID) can be used for virtually all the different classes of compounds by choosing the appropriate characteristic ion or ions. A summary of the important characteristic ions that have been used for this type of analysis and for kerosene oil are given in Table 3.1 (Philp, 1985). The n-alkanes and isoprenoids, iC_{14}, farnesane (iC_{15}: 2,6,10-trimethyldodecane) and 2,6,10-trimethyl-tridecane (iC_{16}), were targeted using the SIM ions and were further confirmed using the national institute of standards and technology (NIST) library. It is accepted within the industry to express the isoprenoid iC_{14} without the international union of pure and applied chemistry (IUPAC) nomenclature system name (Wang, 2010).

The NIST library search on the Agilent GC/MS software allows for a comprehensive search against a reference database of mass spectral compounds. The NIST search works by comparing the ion count between spectra and determining the closest match. The search displays the compound structure and library fragmentogram. The search works on fit (similar to NIST), reverse fit (what major peaks are missing) and purity (based on the amount of background noise per spectrum) (Good, 2002). Anything over 80% is considered a good match.

Table 3.1 Characteristic Ions (Philp, 1985; Wang, et al., 2006b)

Compound Class	SIM Ion (m/z)	Secondary SIM Ion (m/z)
n-Alkane	85	71, 99
Acyclic isoprenoids	183	113, 127

3.10 n-Alkane SIM Analysis of Kerosene

In situations where weathering has not altered the n-alkane pattern significantly, certain diagnostic information can be revealed by the shape of the unresolved complex envelope or the relative abundance of the n-alkane to the isoprenoids (Stout et al., 2002b). The depletion of n-alkanes relative to the isoprenoids is the basis for the age estimation of release within this study. It is on this basis that the individual peaks in the profile obtained from the kerosene chromatography are clearly identified using SIM GC/MS analysis and NIST library comparisons.

Using the Chemstation software the characteristic ion for n-alkanes (m/z 85) was selected and a SIM chromatogram was plotted. The SIM n-alkane (m/z 85) chromatogram was compared to the total ion chromatogram (TIC) for kerosene (Figure 3.7). The retention time for each peak was noted as this will remain constant under similar gas chromatographic conditions. The retention times were compared to the Florida mix n-alkane standard which was analysed under similar conditions and it was found to be consistent within the nonane (nC_9) to hexadecane (nC_{16}) n-alkane range.

The top chromatogram (Figure 3.7) represents the total ion chromatogram for kerosene. It contains all of the ions acquired during the GC/MS analysis. The bottom chromatogram (Figure 3.7) represents the isolation of the m/z 85 ions characteristic for n-alkanes using the SIM mode on the mass spectrometry Chemstation software. The distributions of the m/z 85 ion peaks show a close correlation when compared to the total ion chromatogram (TIC) trace (Figure 3.7). By making this comparison, it can be seen that the major peaks in the TIC chromatogram correspond well with the major peaks in the fragmentogram plot of m/z 85 and are therefore likely to be a series of n-alkanes.

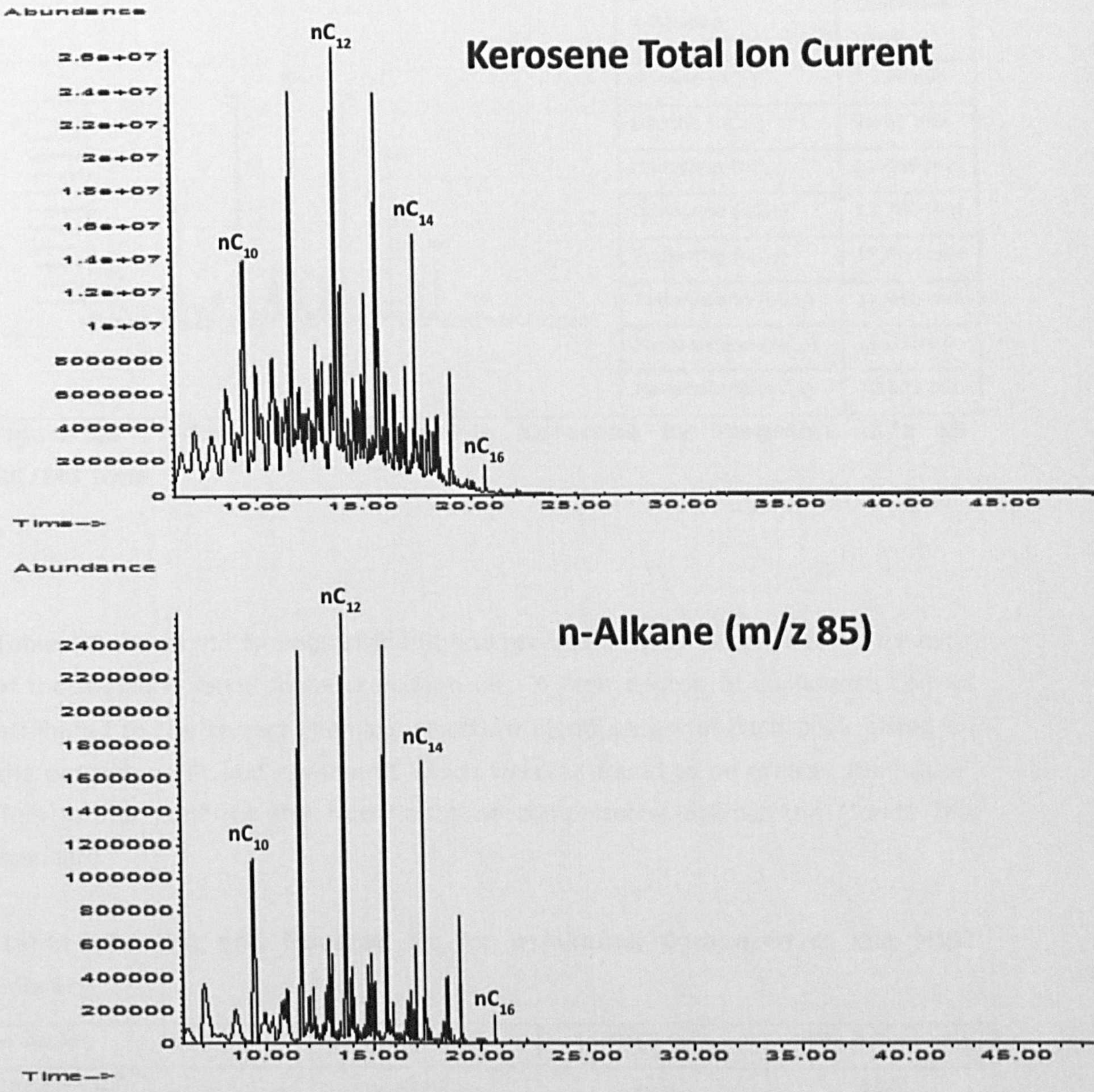
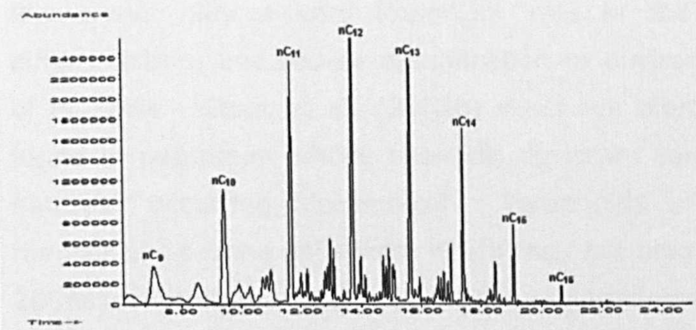


Figure 3.7 Kerosene Total Ion Current Compared to m/z 85

In order to confirm the assignment of n-alkanes in kerosene the fragmentation spectral data for each of the individual n-alkanes in the homologous series was obtained and compared to the NIST library (Figure 3.8).



n-Alkane	Retention Time
Nonane (nC_9)	7.134 min
Decane (nC_{10})	9.469 min
Undecane (nC_{11})	11.689 min
Dodecane (nC_{12})	13.760 min
Tridecane (nC_{13})	15.660 min
Tetradecane (nC_{14})	17.445 min
Pentadecane (nC_{15})	19.076 min
Hexadecane (nC_{16})	20.672 min

Figure 3.8 n-Alkane Distribution in Kerosene by Targeting m/z 85 GC/MS Ions

Table 3.2 shows the findings of the fit and reverse fit NIST Library search for each of the n-alkane found in the kerosene oil. A high degree of confidence can be attributed to the correct chemical structure identification of each peak based on the percentage fit and reverse fit which were all found to be greater than 80%. This further confirms the retention time comparisons against the Florida mix standard.

Table 3.2 Fit and Reverse Fit for n-Alkanes Compared to the NIST Library

n-Alkane	Retention Time	NIST Fit	NIST Reverse Fit
Nonane (nC_9)	7.134 min	80%	87%
Decane (nC_{10})	9.469 min	80%	95%
Undecane (nC_{11})	11.689 min	90%	94%
Dodecane (nC_{12})	13.760 min	86%	92%
Tridecane (nC_{13})	15.660 min	85%	93%
Tetradecane (nC_{14})	17.445 min	91%	96%
Pentadecane (nC_{15})	19.076 min	89%	92%
Hexadecane (nC_{16})	20.672 min	87%	93%

3.11 Identification of Biomarker Isoprenoids in Kerosene by SIM GC/MS

Biomarkers play a very important role in the characterisation, correlation, differentiation, and source identification in environmental forensic investigations of oil spills. Stout et al. (2002b) describes biomarkers as organic compounds found in petroleum whose chemical structure can be unequivocally linked to a naturally occurring biochemical. Terpenoids are classified according to the number of isoprene units from which they are biogenetically derived (Wang et al., 2006b). Farnesane (iC_{15}: 2,6,10-trimethyldodecane) (Figure 3.9) is an example of a regular acyclic isoprenoid consisting of three head-to-tail linked isoprene units. iC_{14} and 2,6,10-trimethyl-tridecane (iC_{16}) are two of the other isoprenoids found in kerosene.

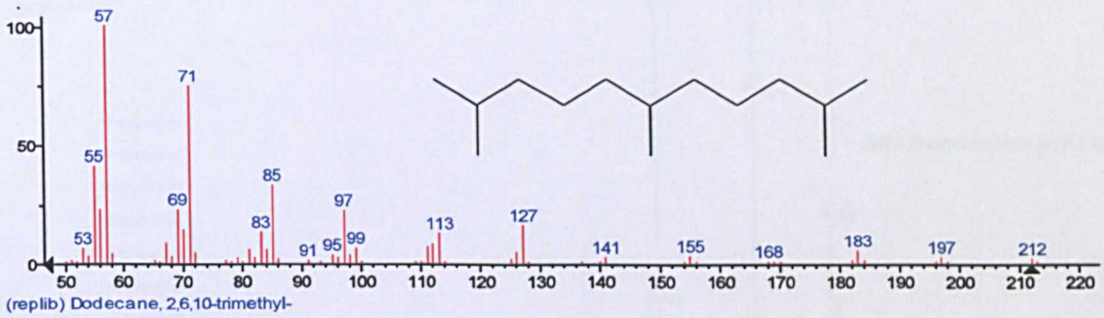


Figure 3.9 Farnesane (iC_{15} : 2,6,10-trimethyldodecane)

The three isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) were targeted using the characteristic ions for these isoprenoids (m/z 183) in the kerosene product sample. The resulting fragmentogram (Figure 3.13) clearly identifies the three isoprenoids eluting between dodecane and pentadecane. The top TIC chromatogram (Figure 3.10) is of the data acquisition of a kerosene sample by GC/MS. The bottom chromatogram (Figure 3.10) is the SIM targeted m/z 183 chromatogram for the main ion found in isoprenoids. The isoprenoid iC_{14} was found to elute before the n-alkane tridecane (nC_{13}). Farnesane (iC_{15}) was identified to elute before the n-alkane tetradecane (nC_{14}) with 2,6,10-trimethyl-tridecane (iC_{16}) eluting before pentadecane (nC_{15}).

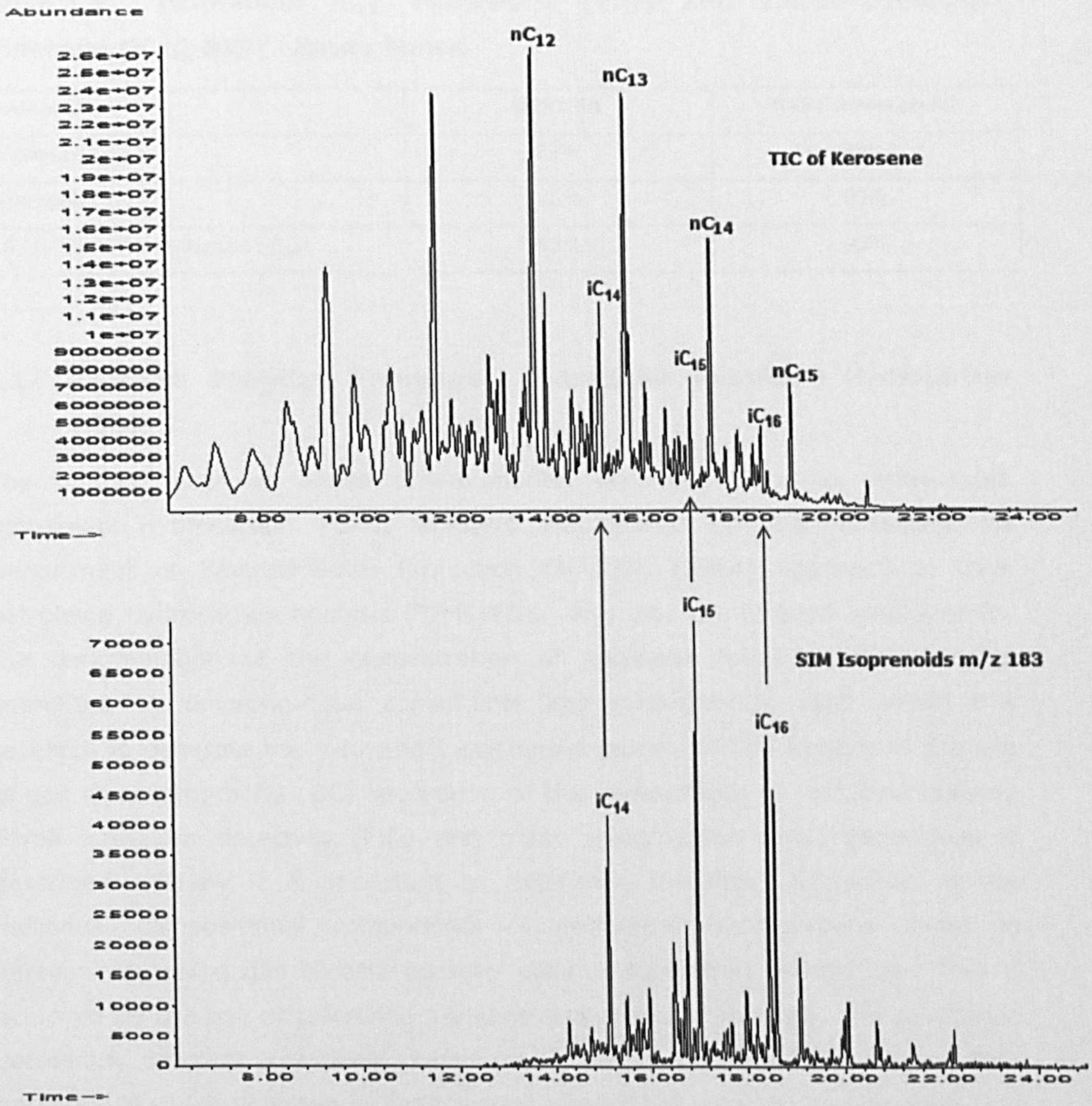


Figure 3.10 TIC and SIM Chromatogram of Kerosene Identifying iC_{14}, Farnesane (iC_{15}) and 2,6,10-Trimethyl-tridecane (iC_{16}) Isoprenoids using m/z 183

The fragmentogram for the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) were compared against the NIST database library and the percentage fit is shown in Table 3.3. A high degree of confidence can be attributed to the correct chemical identification of each peak based of the percentage fit and reverse fit which were all found to be greater than 80%.

Table 3.3 Isoprenoid iC_{14}, Farnesane (iC_{15}) and 2,6,10-Trimethyl-tridecane (iC_{16}) NIST Library Match

n-Alkane	NIST Fit	NIST Reverse Fit
Isoprenoid (iC_{14})	82%	85%
Farnesane (iC_{15})	85%	89%
2,6,10-Trimethyl-tridecane (iC_{16})	82%	90%

3.12 Research Analytical Procedure - Extractable Petroleum Hydrocarbon (EPH)

The method used at Jones Environmental Forensics Ltd., for extractable petroleum Hydrocarbon (EPH) analysis was based on the Massachusetts Department of Environmental Protection (MADEP) (2004) approach to total petroleum hydrocarbon analysis (TPHCWG). This was the method employed for the determination of the concentration of kerosene found in the samples submitted by environmental consultants and subsequently used within this research to generate the n-alkane / isoprenoid ratios. Within Chapter 4 the use of gas chromatography (GC) separation of the components of kerosene utilising flame ionisation detection (FID) and mass spectrometry (MS) techniques is described. Firstly it is important to determine the order of elution of the individual compositional components of petroleum hydrocarbons found in kerosene by using gas chromatography column separation techniques. This is achieved by the use of reference n-alkane and aromatic mixture, and purchased petroleum products (gasoline, kerosene, diesel, lubrication oils etc.). This approach is widely adopted by laboratories around the world to demonstrate that their methods are fit for purpose.

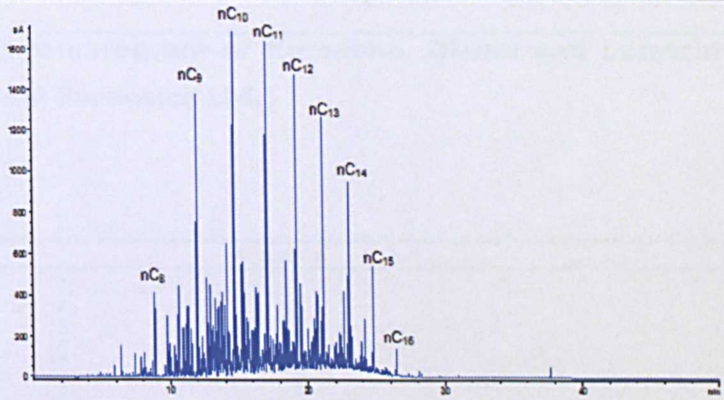
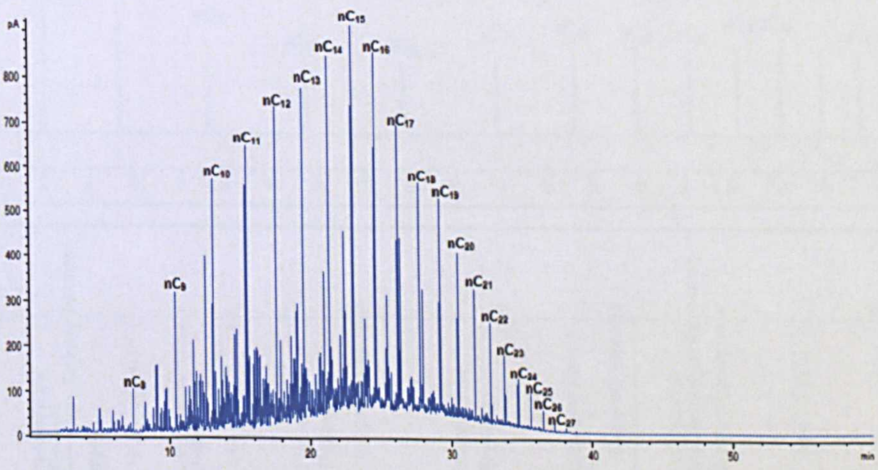
The EPH method used within this research at Jones Environmental Forensics Ltd., complied with the criteria set out by the laboratory quality standard, international organization for standardization general requirements for the competence of testing and calibration laboratories (ISO17025) which was satisfactorily annually audited externally by the United Kingdom Accreditation Service (UKAS). To ensure the ongoing performance of the EPH method the laboratory complied with the following criteria around the time in which the samples used in this research were analysed. The typical criteria used are:

- A surrogate spike added at the extraction stage to all soil samples with an acceptable recovery range of 70 – 130%;
- Extracted blank samples run with every batch;
- Quantification performed by means of an external standard technique, against a six point calibration;
- A calibration check standard analysed at the start and end of every run of samples in order to verify the linearity of the six point external calibration;
- External calibration within a range of concentration found within this study with a linearity coefficient of determination (R^2) of 0.995 or better;
- Analytical quality control (AQC) samples every twenty samples in a batch;
- Certified Reference Material (CRM) analysed within each batch;
- Acceptable performance in external proficiency testing (PT) schemes; and
- System suitability performance monitoring including peak asymmetry and peak separation.

Appendix 1 details the separation and instrumental conditions used for the extractable petroleum hydrocarbon method.

3.12.1Research Fingerprinting - Chromatographic Profiling of Kerosene

The characterisation of the chromatographic profile (unique fingerprint) of kerosene is evaluated in Chapter 4. This work was undertaken at Jones Environmental Forensics Ltd. The order of elution and retention times of individual n-alkanes and aromatics on a GC chromatographic system is necessary to ensure correct identification of the carbon ranges of petroleum products when analysed on these systems. This was achieved with the use of a ChemService standard. This standard was prepared to establish the chromatographic profile of n-alkanes and polycyclic aromatic hydrocarbons typically found in kerosene and other extractable petroleum hydrocarbon products. This profiling of n-alkanes and PAHs is important as it provides consistency when reviewing chromatograms over time. The instrumental chromatographic conditions are unlikely to alter significantly over time. However, to ensure this consistent approach is achieved routinely the retention-time-locking standard *Florida total petroleum hydrocarbon standards mixture* (TPH-7RPM), a mixture of total petroleum hydrocarbon solution containing a range of n-alkanes from octane (nC_8) to tetracontane (nC_{40}) is used. The chromatogram obtained from the analysis of this standard is illustrated in Figure 3.11. The order of elution and retention time for each n-alkane from this chromatogram can be determined and compared to petroleum products such as kerosene, diesel and lubrication oil (Figure 3.11). The order of elution and retention time of the aromatic species was achieved by the analysis of a polycyclic aromatic hydrocarbon (PAH) standard mixture (Figure 3.12). The analysis of a kerosene product described in Chapter 4 produced an n-alkane distribution for kerosene in the carbon range octane (nC_8) to hexadecane (nC_{16}), this carbon range was determined by the use of this reference Florida mix retention-time-locking standard described here.

Petroleum Product	Extractable Petroleum Hydrocarbon Chromatogram
Kerosene	
Diesel	

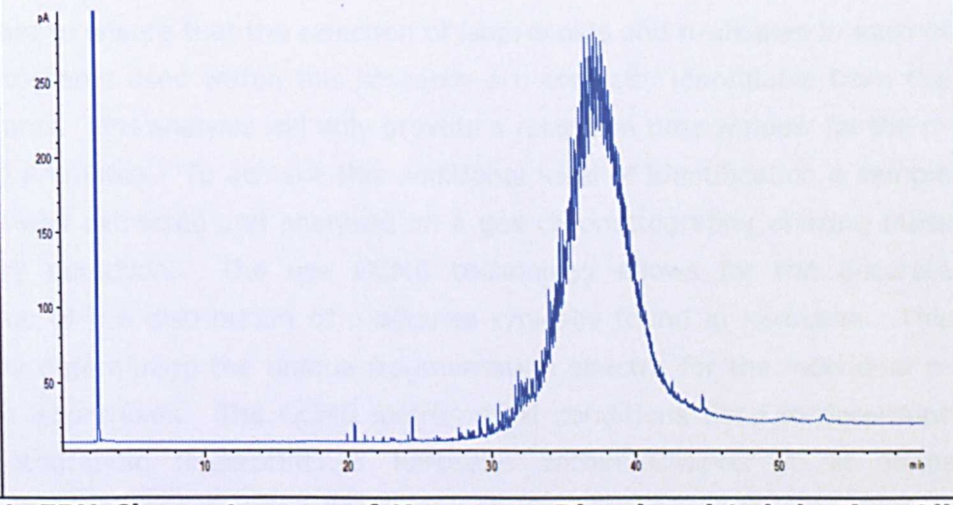
Petroleum Product	Extractable Petroleum Hydrocarbon Chromatogram
Lubrication Oil	

Figure 3.11 EPH Chromatogram of Kerosene, Diesel and Lubrication Oil (Jones Environmental Forensics Ltd.)

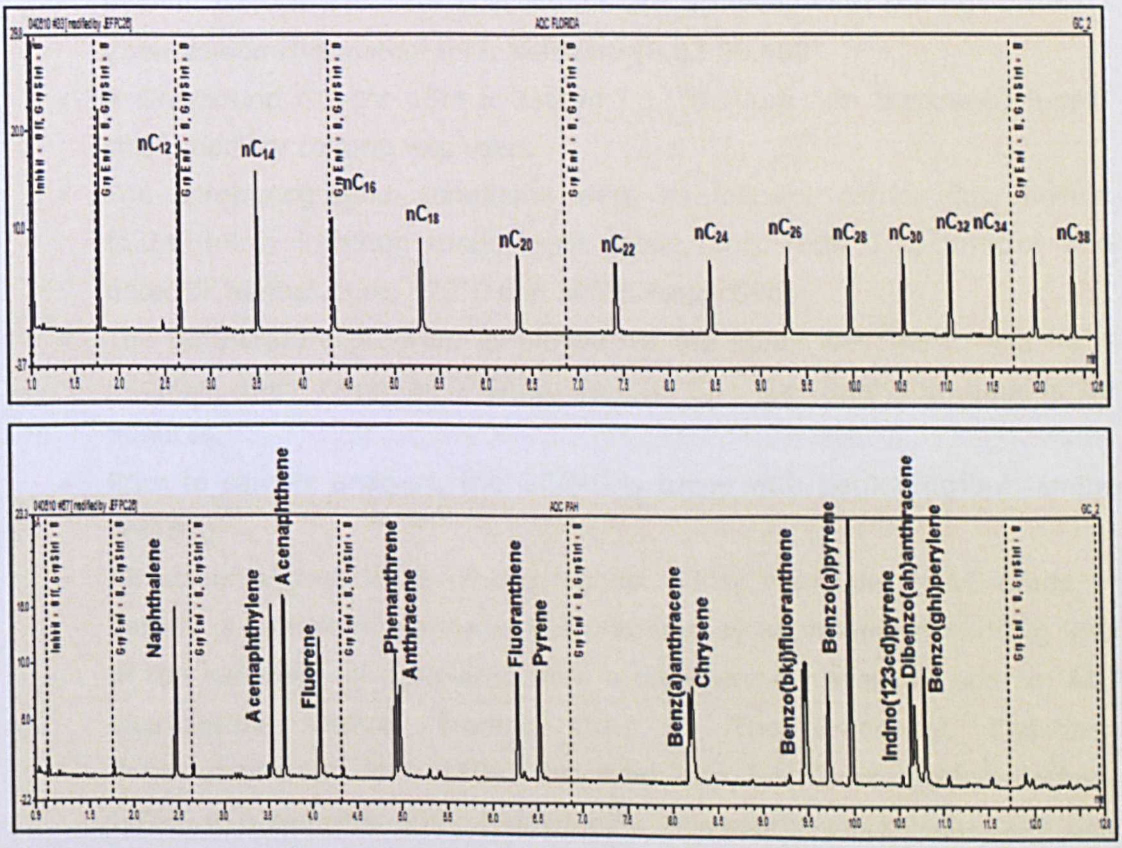


Figure 3.12 TPH Florida and PAH Standard Mixture

3.12.2 Chromatographic Profiling of the Isoprenoids and n-Alkanes in Kerosene

It is important to ensure that the selection of isoprenoids and n-alkanes in each of the chromatograms used within this research are correctly identifiable from the chromatograms. EPH analysis will only provide a retention time window for the n-alkanes and aromatics. To achieve this additional level of identification a sample of kerosene was extracted and analysed on a gas chromatography utilizing mass spectrometry detection. The use GCMS technology allows for the accurate determination of the distribution of n-alkanes typically found in kerosene. This was done by determining the unique fragmentation spectra for the individual n-alkanes and isoprenoids. The GCMS instrumental conditions used to determine the chromatographic fingerprint of kerosene within Chapter 4 at Jones Environmental Laboratory are as follows.

- An analysis of kerosene was performed on an Agilent 7890 GC coupled with an Agilent 5975B inert mass selective detector (MSD).
- System control and data acquisitions are achieved with the Agilent MSD Chemstation chromatographic software (E.02.00.493).
- A Greyhound GC-1ht 15m x 250µm i.d. (0.10µm film thickness) fused-silica capillary column was used.
- The chromatographic conditions were as follows: carrier gas, helium (1.2ml/min); injection mode, split mode, split ratio 1:1; injector and detector temperature, 320°C and 300°C respectively.
- The temperature program employed for the study was: 45°C hold for 4 minutes, then ramp at 7°C/min to 300°C. The total run time is 40 minutes.
- Prior to sample analysis, the GC/MS is tuned with perfluorotributylamine (PFTBA).
- All solvents used were of the highest purity available, HPLC grade or better. A sample of kerosene was prepared by approximately adding 50µl of the kerosene oil purchased from a commercially available source; ACT (Agricultural Central Trading Ltd., 90 The Broadway, Chesham, Buckinghamshire, HP5 1EG), dissolved into 1.5mls of dichloromethane (DCM) in a 2ml clear crimp topped vial. This source was selected as it best represented and reflected the typical spilled product found within this study.

Wang and Stout, 2007 describe the use of mass spectrometer detectors (MS) as the most powerful detector for a gas chromatograph (GC) due to its high sensitivity, specificity and its capability to elucidate compound structures. The fragmentation mass-to-charge ratio (m/z) for n-alkanes (m/z 85, 71 and 99) and isoprenoid (m/z 183, 113 and 127) is described in Chapter 4. Using a combination of retention time and the selective mass spectral fingerprints the target n-alkanes, dodecane (nC_{12}), tridecane (nC_{13}) and tetradecane (nC_{14}) and isoprenoids (iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) were identified and confirmed (Figure 3.13).

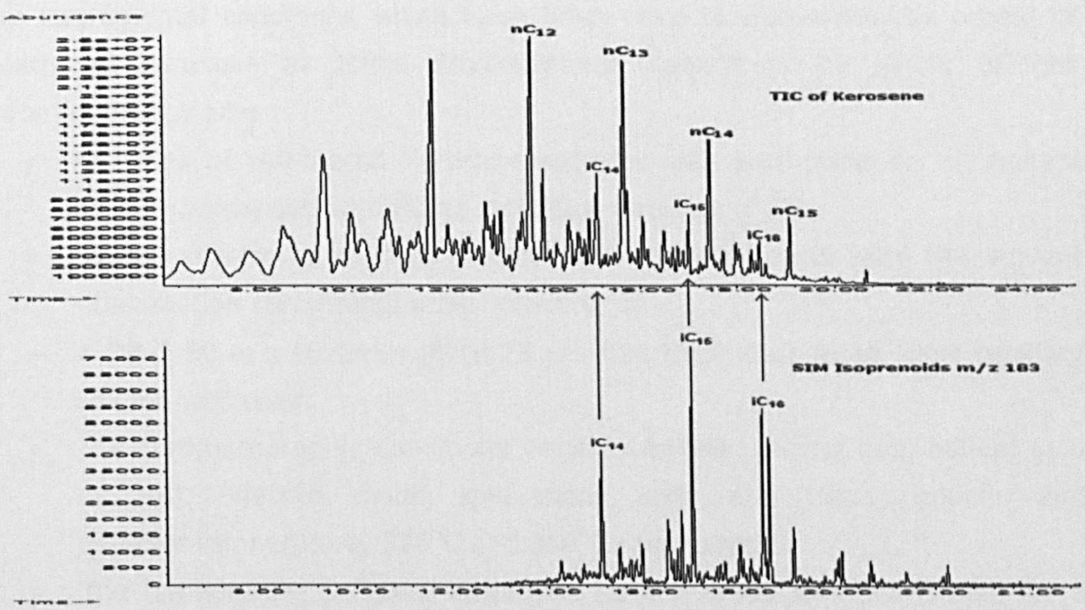


Figure 3.13 Total Ion Chromatogram and Selective Ion Monitoring Chromatogram of Kerosene Identifying iC_{14}, Farnesane (iC_{15}) and 2,6,10-Trimethyl-tridecane (iC_{16}) Isoprenoids using m/z 183

3.12.3Research Experiment - Weathering Profile of Kerosene

The purpose of the laboratory based weathering experiment was to determine if the n-alkanes to isoprenoid, dodecane (nC_{12}) : iC_{14}, tridecane (nC_{13}) : farnesane (iC_{15}) and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) showed preferential depletion of the n-alkanes over their neighboring isoprenoids. The experiment aims to evaluate whether a linear relationship exists between the n-alkane and isoprenoid as described in the Christensen and Larsen (1993) study of the depletion of heptadecane (nC_{17}) / pristane in diesel. In a number of graduated glass vials, to each, 4mls of neat kerosene was placed on a hot plate and each vial was placed under a constant stream of nitrogen over a period of twenty four hours. The 4mls of kerosene was reduced to 25%, 50% and 75% of their initial volume. As each of the evaporation levels was achieved they were removed from the hot plate and stream of nitrogen, stored and analysed. The experiment was performed in triplicate, the neat kerosene and the laboratory weathered kerosenes were analysed by whole oil gas chromatography. Whole oil analysis (ISO17025 UKAS accredited) which was performed at Jones Environmental Forensics ltd. (Appendix 1) is a petroleum geochemistry method which allows for the determination of the bulk characteristics, distillation range and the banding of a pure product by GC/FID analysis. The carbon range for this method is hexane (nC_6) to triacontane plus (nC_{35+}) where kerosene will elute. The instrumental conditions which have been used to determine the profile of weathered kerosene at Jones Environmental Laboratory by whole oil gas chromatography are:

- Analyses of weathered kerosene samples was performed on an Agilent 6850 GC equipped with flame ionisation detector (FID).
- System control and data acquisitions was achieved with the Agilent Chemstation chromatographic software.
- A DB-1 50 m x 0.25mm ID (0.25 μm film thickness) fused-silica capillary column was used.
- The chromatographic conditions were as follows: carrier gas, helium (1.0 ml/min); injection mode, split mode, split ratio 150:1; injector and detector temperature, 320°C and 350°C respectively.
- The temperature program employed for the study was: 40°C hold for 5 minutes, then ramp at 8°C/min to 200°C and hold for 1 minute followed by a ramp at 10°C/min to 340°C and a final hold for 20 minutes.

A normalized histogram was generated to demonstrate the degree of weathering of the lighter n-alkanes. This was achieved by the use of the neat kerosene which was used as the reference oil. All of the n-alkanes peak areas were normalized to the total of the n-alkanes peak area. This allowed for a visual representation of the depletion of the individual n-alkanes compared to the neat un-weathered kerosene. Using linear regression the ratio of the n-alkane to the isoprenoid was obtained for dodecane (nC_{12}) / iC_{14}, tridecane (nC_{13}) / farnesane (iC_{15}) and tetradecane (nC_{14}) / 2,6,10-trimethyl-tridecane (iC_{16}) from each of the evaporation levels and the neat kerosene to determine if there is a relationship between the depletion of the n-alkane relative to the recalcitrant isoprenoid. This was evaluated by the correlation coefficient of the linear regression line plotted. Chapter 4 presents the findings of this weathering study.

3.13 Research Question 2 – Development of Analytical Protocol For Chromatogram Interpretation

The development of an analytical protocol for chromatogram interpretation was required due to the high number of samples likely to be reviewed within this research (6,100). It became apparent quickly that it was necessary to develop an analytical method for the rapid extraction of isoprenoid and n-alkane data content within each of the chromatograms under review. A guide (Appendix 2) was developed to speed up the extraction of the numerical data associated with peak height and peak area for each of the n-alkanes and isoprenoids reviewed within this research. The n-alkanes selected for review following the analysis of kerosene and weathering experiment in Chapter 4, were dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) and the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}). The purpose of this guide was to document a stepwise approach to data gathering to allow laboratory technicians in the future to reproduce this data collection process when the kerosene age estimation model is brought to commercialization. The GCFID and GCMS interface software which was used was an Agilent Technologies Chemstation software. Agilent Chemstation software is a chromatography data management system which acts as an interface between gas chromatography systems and the laboratory technician. The Chemstation software controls the analytical method employed by the GC instrument to ensure a particular separation is consistently performed. It contains all parameters for instrument control, data acquisition and evaluation, including integration, quantification and reporting. During analysis of a sample a data file was generated and stored by the Chemstation software. The information contained within this data file can be used to extract information such as peak areas or peak height which is necessary for the kerosene age estimation model.

3.13.1 Research Data Collection - Chemstation Software Peak Area Measurement

The precise measurement of the correct peak within the chromatogram was critical to establishing a reproducible ratio for the n-alkanes to isoprenoids across the sample database. The peak area measurement was achieved using a manual integration option available from the Chemstation software. Manual integration allows the user to integrate selected peaks or groups of peaks within the chromatogram. It allows the user to define the start and stop points for the peak of interest. After the selection of the appropriate peak a baseline-to-baseline

(BB) (Figure 3.14) integration can be achieved and the software will calculate and report a peak area and peak height for the selected peak (Agilent, 2009).

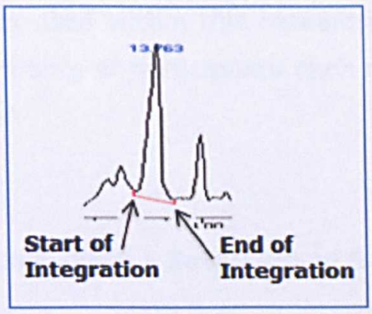


Figure 3.14 Peak Baseline-to-baseline Integration

3.14 Research Question 3 – Development of a Kerosene Database

The third objective was to develop a database of chromatograms and associated meta data obtained from the analysis of kerosene spill samples (soil and water) received for analysis from 2009 to 2011 at Jones Environmental Forensics Limited Laboratory. These samples have been submitted for analysis by environmental consultants working on domestic home heating oil releases in Ireland and the United Kingdom (Chapter 5). Following on from this an evaluation was undertaken to analyse the database of isoprenoid and n-alkane data of kerosene spill samples (soil). This was conducted to evaluate the environmental factors (weathering and biodegradation) affecting isoprenoids and n-alkane ratios in post-spill kerosene in soil in order to accurately date a spill event (Chapter 6).

3.14.1 Research Dataset Origin - Environmental Consultants Firms

Prior to commencing formal data collection, preliminary research was conducted with several senior consultants within different consultancy firms to establish which firms are likely to work on kerosene releases. The research work presented in Chapter 5 focuses on a detailed investigation into 6,100 chromatograms. This was narrowed down to 473 chromatograms as these contained the profile expected for a kerosene product. These chromatograms were derived from the extraction of soil samples submitted by environmental consultants to Jones Environmental Forensics Laboratory (JEFL) between 2009 and 2011. Nine different environmental consultancy firms submitted samples within this time period. Due to confidentiality agreements between Jones Environmental Forensics Ltd and each consultancy firm, it was not possible to identify the consultancy firms company name, the project name, reference on the samples, or the location of where the samples was taken. Written agreement was obtained from each of these companies and from Jones Environmental Forensics Ltd., to use these data and methods used within this research. In an effort to maintain this anonymity and confidentiality of participants each consultancy was assigned a unique identification number.

3.14.2Preliminary Data Collection - Selection of Samples for the Database

Of the 6,100 chromatograms reviewed 473 chromatograms had profiles which were characteristic of a kerosene home heating oil contaminant. These were included in the table and evaluated in this research. The kerosene database of 473 samples (Appendix 3) is a table which contains details of:

- The sample number for the chromatogram examined out of the 473 samples submitted,
- The consultant identification number,
- The depth at which the sample was taken,
- The concentration of the EPH analysis,
- The peak area of each of the n-alkanes and isoprenoids,
- The isoprenoid to n-alkane ratio, and
- The release data and the sampling date.

Table 3.4 details the number of samples submitted by each of the environmental consultancy firms.

Table 3.4 Environmental Consultant and Number of Samples Submitted

Consultant Number	Number of Samples
Consultant 1	93
Consultant 2	199
Consultant 3	1
Consultant 4	159
Consultant 5	9
Consultant 6	6
Consultant 7	2
Consultant 8	3
Consultant 9	1
Total	473

Consultancy firm number 2 (199 samples) chose not to partake in the research and therefore all samples from this firm were excluded from the study. Other factors which resulted in the omission of samples were:

- The consultancy firm when contacted were not able to provide a release date for the spill event,
- The release was described as an ongoing release over time rather than a once off release,
- The sample submitted was identified as a validation sample,
- The soil depth was unknown - for example a composite sample at different depths was taken,
- The sample was described as a surface sample, and
- The samples were described as water samples.

A validation sample in this circumstance is described as the final sample taken following a site investigation to demonstrate the effectiveness of the remediation works. Any remediation actions undertaken by the environmental consultant would enhance the depletion of the n-alkane compared to that of the isoprenoid and make this invalid for inclusion in the model.

Once all of the n-alkane and isoprenoid chromatographic data was obtained for the n-alkanes, dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) and the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) from the 473 chromatograms it was necessary to include the other meta data which was contained within the Jones Environmental Laboratory final report for each consultant. The laboratory assigned project number and sample number was recorded during the collection of data from the review of the 473 chromatograms. This allowed for the review of each of the project's final reports. Contained within each of these reports was information such as:

- The consultancy firms company name and project manager contact details,
- The consultants' project name and location of the site under investigation,
- The Jones Environmental Forensics Ltd project and sample numbers,
- Each of the sample references identification for example borehole (BH1) and monitoring well (MW1),
- Date at which the sample was taken by the consultant,
- The sample type for example soil, product or water,
- The depth at which the sample was taken, and

- The UKAS accredited analytical results for the concentration following EPH analysis.

3.15 Research Question 4 – Kerosene Age Estimation Model Equation Generation

This objective was to develop an equation equivalent to the Christensen and Larsen (1993); Kaplan, Galperin, Alimi et al. (1996b) equation used for middle distillate, but based on forensic markers of isoprenoids and n-alkanes present in kerosene, which is applicable to kerosene spills. This is evaluated in Chapter 5 where the data base was tested using a variety of regression algorithms. The use of statistical algorithms is primarily concerned with how to summarise and interpret variables (DeCoster, 1998). These variables include volatilization, biodegradation and chemical alterations of the released kerosene following release into soil. The ultimate objective of the statistical trend line analysis was to identify and develop a model that will accurately predict *y* as a function of a set of predictor variables *x* taking into account all of the environmental influences (evaporation and biodegradation) at the spill location.

3.15.1 Descriptive Statistics

The two models described in Chapter 2 for the age estimation of release of diesel in the Christensen and Larsen (1993); Kaplan, Galperin, Alimi et al. (1996b) and Hurst and Schmidt (2005) studies employed the use of a linear equation $y = \beta x + \alpha$ or y = mx +c where *y* is a dependent variable, x is an independent variable, α is the linear intercept, β is the linear slope (Walpole and Myers, 1989; Rong, 2000). For this research it was hoped that a similar equation may be applied to the n-alkanes and isoprenoids in kerosene.

From the kerosene database presented in Chapter 5 each of the ratios of dodecane (nC_{12}) : iC_{14}, tridecane (nC_{13}) : farnesane (iC_{15}) and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release the R^2 value and equation was calculated for the following regression models using Microsoft excel:

- Linear Regression Model,
- Exponential Regression Model,
- Power Regression Model,

- Logarithmic Regression Model, and
- Polynomial Regression Model.

3.15.2 Validating the Model

Cross-validation as a model validation tool is described in Chapter 2. In Chapter 5 each observation within the kerosene dataset was left out once and predicted by the model generated from the rest of the data. The procedure eventually results in a complete set of predicted values, each of which was generated by a model independent of the predicted value (Michaelsen, 1987). For each of the regression equation models listed in *3.15.1 Descriptive Statistics,* a cross validation evaluation was undertaken to determine the predicted value for each observation, the residual and the mean squared error (MSE) for the observations within the model. The predicted value was determined on the bases of *k*-fold or leave-one-out cross validation. Each individual observation was removed from the data set once, and the predicted value for this omitted observation was then determined by the remaining observations within the dataset (Figure 3.15).

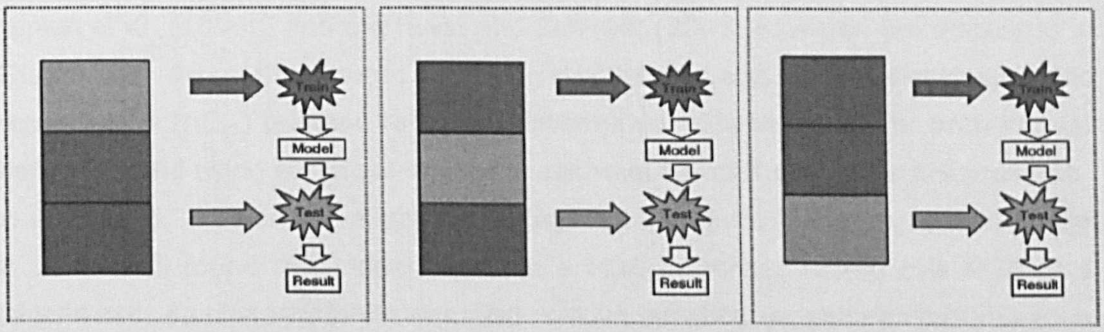


Figure 3.15 Cross-Validation (Refaeilzadeh et al., 2008)

The residual was determined as the difference between the number of days following release (Release Days) and predicted value by the model under validation. The equation described by Michaelsen, (1987) is as follows.

$$r_{(i)} = y_i - \hat{y}_{(i)} \text{ (Michaelsen, 1987)}$$

Where y_i and $\hat{y}_{(i)}$ are the observed and predicted values. The notation (i) indicates that data for i were not used in fitting the model that generated the prediction $\hat{y}_{(i)}$ (Michaelsen, 1987).

Equation 4 Residual Equation

The mean of squared error of the residuals (MSEr) was the mean squared error for the model data.

$$\text{MSE}r = \frac{\text{SSE}_r = \sum_{i=1}^{n_r} (r_{(i)})^2}{n_r} \text{ (TUOA, 2012)}$$

Equation 5 Mean of Squared Error of the Residuals

The closer the prediction to the actual data will result in a smaller value for the MSEr. (TUOA, 2012).

3.15.3 Cross Validation Reassessment Based on MSE

The Christensen and Larsen 1993 study using the equation later generated by Kaplan et al. (1996b) and the Hurst and Schmidt (2005) equation are discussed in Chapter 2. From the figures for both studies the age of the diesel spill and heptadecane (nC_{17}) pristane ratio was determined. These values for both studies were assessed using the cross-validation technique and the findings are presented in Chapter 5. The cross validation findings for the Hurst linear regression model in Chapter 5 found the model reported a MSE of three. Using this MSE as a benchmark, a cross validation exercise was undertaken on each of the individual models (Exponential, Linear, Power and Polynomial) by selectively removing sample numbers and recalculating the model until each model achieved a MSE of three.

An analysis of variance (ANOVA) and percentage relative standard deviation (%RSD) statistical tests was undertaken on the data for each model before and after elimination of samples to evaluate if an improvement was made. A plot of the known number of days following release versus the predicted number of days following release for each model generated an R^2 value which was used as a measure of the models predictive performance.

3.16 Research Question 5 – Kerosene Age Estimation Model Conditions of Use

The criteria constraining the conditions within which the kerosene spill ageing equation can be relied upon was discussed in Chapter 6. The criteria established were generated from the discussion and findings of each of the aims and objectives outlined in analysis of kerosene and weathering experiment chapter 4, the results chapter 5 and the conclusions drawn from the discussion in Chapter 6.

4.0 Chapter 4: Analysis of Kerosene and Weathering Experiment

The pollution of soils and groundwater by kerosene spills is of major concern to domestic householders and their insurers in the UK and Ireland (Busby, Custy and Woolley, 2001; EA, 2010; NIEA, 2010; and SEPA, 2010). Kerosene spills may persist in the soil as a source of hazardous hydrocarbons for a long time, but not as long as diesel (up to 5 years) because of the low solubility and the moderate to low volatility of kerosene constituents (Dror, Gerstl and Yaron, 2001). Generally, hydrocarbons in kerosene biodegrade significantly under aerobic conditions, provided that sufficient amounts of essential nutrients are present (Shabir, Afzal, Anwar et al., 2008). Other variables include:

1. The specific composition of spilled kerosene (Jones, 2003).
2. The soil depth profile and moisture content before contamination and resultant leaching pattern which determines volatilisation and redistribution with depth of spilt kerosene (Dror et al., 2001).

The redistribution of kerosene's constituents can lead to a continuing change in the composition of the spill mixture in the subsurface environment (Wang, Fingas, Blenkinsopp et al., 1998). A variety of laboratory techniques are available for the forensic analysis of these hydrocarbons (Alimi et al., 2003). For this study, gas chromatography coupled with flame ionisation detection and mass spectrometry (GC/MS) is employed to identify the main components of kerosene which are likely to alter after a spill and can subsequently be used to determine the age of the spill.

4.1 Evaporation Weathering Profile of Kerosene Oil

When petroleum products are accidently released into the environment, whether on water or land, they are immediately subject to a wide variety of changes in physical and chemical properties that in combination are termed weathering (Wang and Fingas, 2006a). Petroleum attenuation in soil is predominantly governed by:

- Evaporation, occurring when petroleum is in contact with air and soil, causing mainly the lighter end petroleum constituents to volatilise;

- Leaching, also known as dissolution or water washing, in which petroleum constituents dissolve into water; and .
- Biodegradation, the digestion of petroleum constituents by microbes (Oudijk, 2009).

Evaporation is the most important weathering process in terms of environmental impact on the spilled kerosene. This process during the early stages of an oil spill can be responsible for the removal of a large fraction of the oil including the more toxic, lower molecular weight components. Evaporation in the short term is the single most important and dominant weathering process (Wang and Fingas, 2006a). Major factors influencing the rate of evaporation which affects composition and physical properties of the oil are wind velocity, and water temperature (Zhu, Venosa, Suidan et al., 2001).

Kaplan et al. (1997) found that weathering could be divided into a number of progressive stages;

- Abundant n-alkanes;
- Light-end n-alkanes removed;
- More than 90% of n-alkanes removed;
- Alkylcyclohexanes and alkylbenzenes removed; and
- Isoprenoids reduced.

4.2 Experimental Evaporation Weathering of Kerosene in the Laboratory

Whole oil GC/FID analysis follows an ISO17025 UKAS accredited procedure for the determination of the bulk characteristics of oils by GC/FID. The chromatogram obtained serves as a fingerprint of the sample components and allows the determination of the bulk characteristic of the weathered samples.

The instrumental conditions which have been used to determine the profile of weathered kerosene at Jones Environmental Laboratory are as follows:

- Analyses of weathered kerosene samples were performed on an Agilent 6850 GC equipped with flame ionisation detector (FID);
- System control and data acquisitions are achieved with the Agilent Chemstation chromatographic software;
- A DB-1 50m x 0.25mm ID (0.25μm film thickness) fused-silica capillary column was used;

- The chromatographic conditions were as follows: carrier gas, helium (1.0 ml/min); injection mode, split mode, split ratio 150:1; injector and detector temperature, 320°C and 300°C respectively; and
- The temperature programme employed for the study was: 40°C hold for 5 minutes, then ramp at 8°C/min to 200°C and hold for 1 minute followed by a ramp at 10°C/min to 340°C and a final hold for 20 minutes.

Prior to analysis, a reference crude oil (Figure 4.1) was analysed and the chromatogram was assessed for suitability. The heptadecane (nC_{17}) / pristane and octadecane (nC_{18}) phytane ratios were determined and these passed the acceptance criteria for this ISO17025 United Kingdom Accreditation Service (UKAS) accredited method. These substances are used to assess the resolution of the analytical column to ensure that product samples are analysed in a consistent manner (Figure 4.2). If compounds or ratios are used in correlation studies, it is important that the resolution of these compounds is stable and variation is not due to differences in analytical measurement. The resolution between heptadecane (nC_{17}) and pristane is set at 1.50 but in actual fact the resolution is far superior to that and is less than 2% relative standard deviation (RSD) (Figure 4.3).

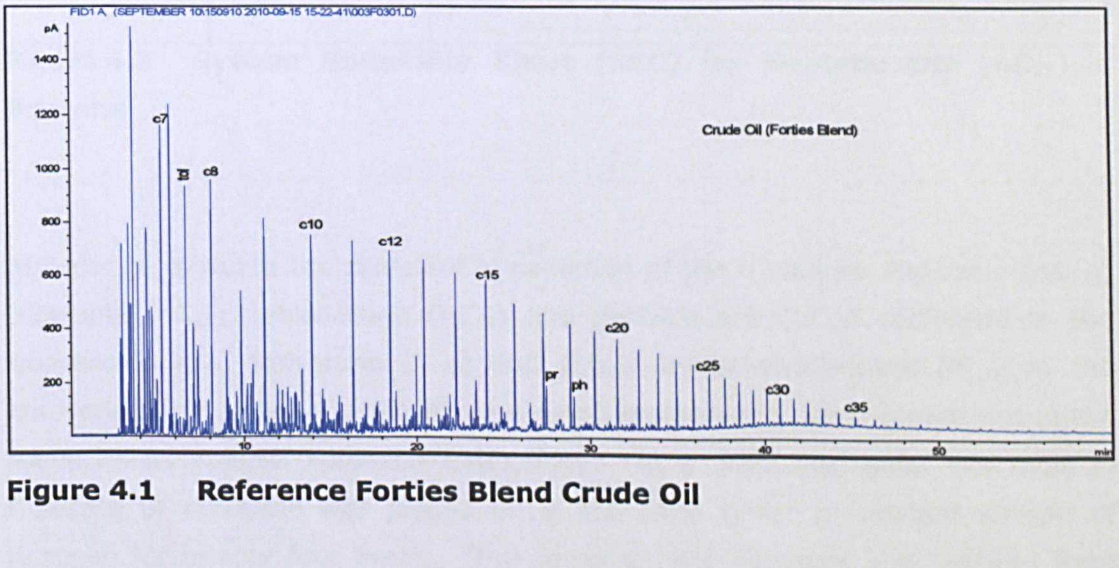


Figure 4.1 Reference Forties Blend Crude Oil

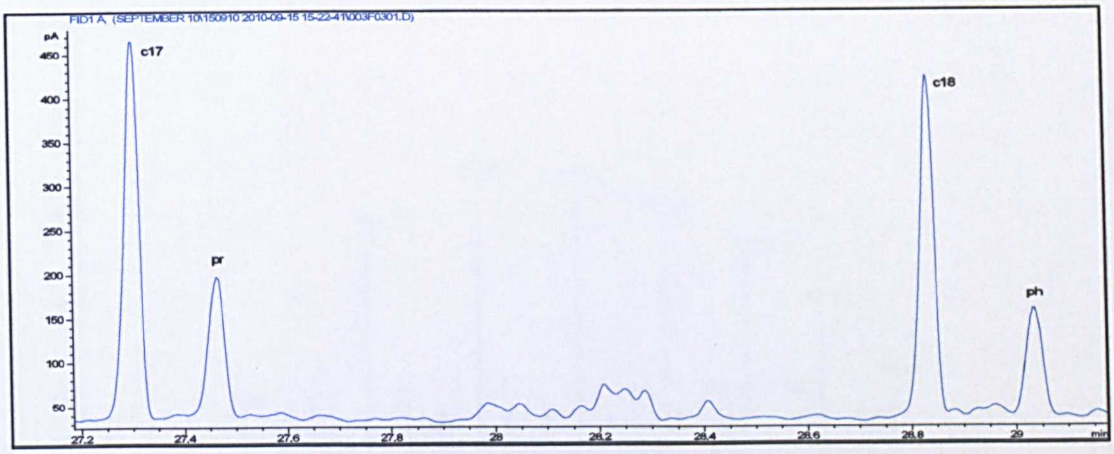


Figure 4.2 Resolution Check for Forties Blend Crude Oil

Between Batch Statistics	
Mean	2.8886
SD	0.0419
Actual Value	
% RSD	1.45
Pass Criteria	1.50
Uncertainty (EU)	-2.8048

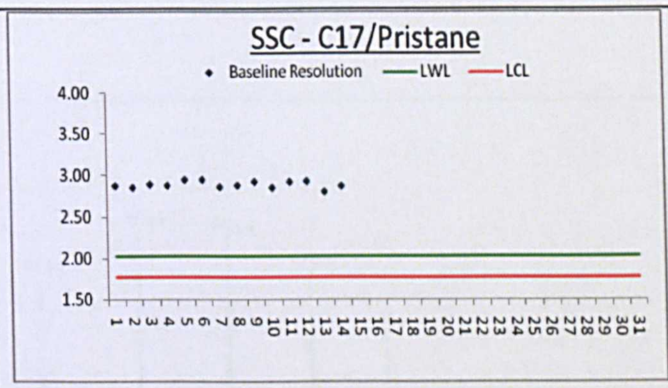


Figure 4.3 System Suitability Chart (SSC) for Heptadecane (nC_{17}) / Pristane

In order to evaluate the preferential depletion of the n-alkanes dodecane (nC_{12}), tridecane (nC_{13}), tetradecane (nC_{14}) and pentadecane (nC_{15}) compared to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil, a series of artificial weathering experiments were carried out in the Jones Environmental Forensics Laboratory. In a graduated glass vial 4mls in triplicate of kerosene was placed on a hot plate under a constant stream of nitrogen for twenty four hours. The samples neat kerosene was reduced from 4mls to 25%, 50% and 75% of their initial volume. The evaporation experiment was performed in triplicate. The reduced volumes and the unaltered neat kerosene oil was then analysed by whole oil GC/FID (Figure 4.4 to Figure 4.7).

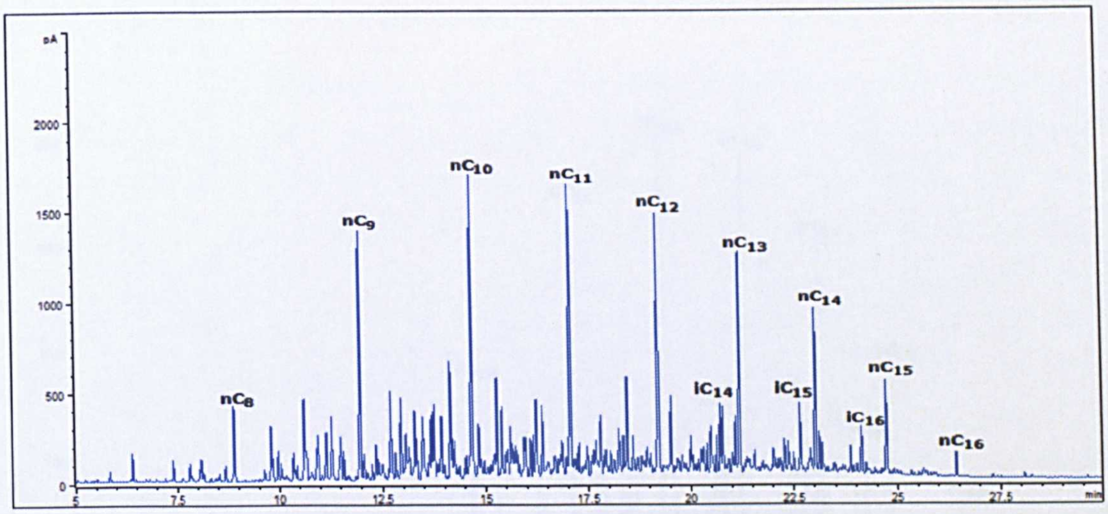


Figure 4.4 Neat Kerosene Oil (Not Evaporated - 100%)

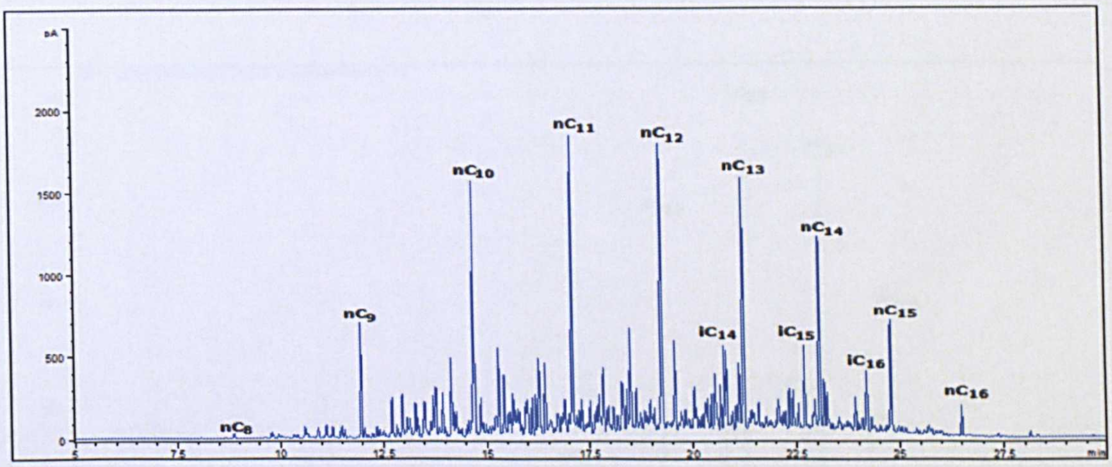


Figure 4.5 Evaporated Kerosene Oil Reduced by 25% (By Volume)

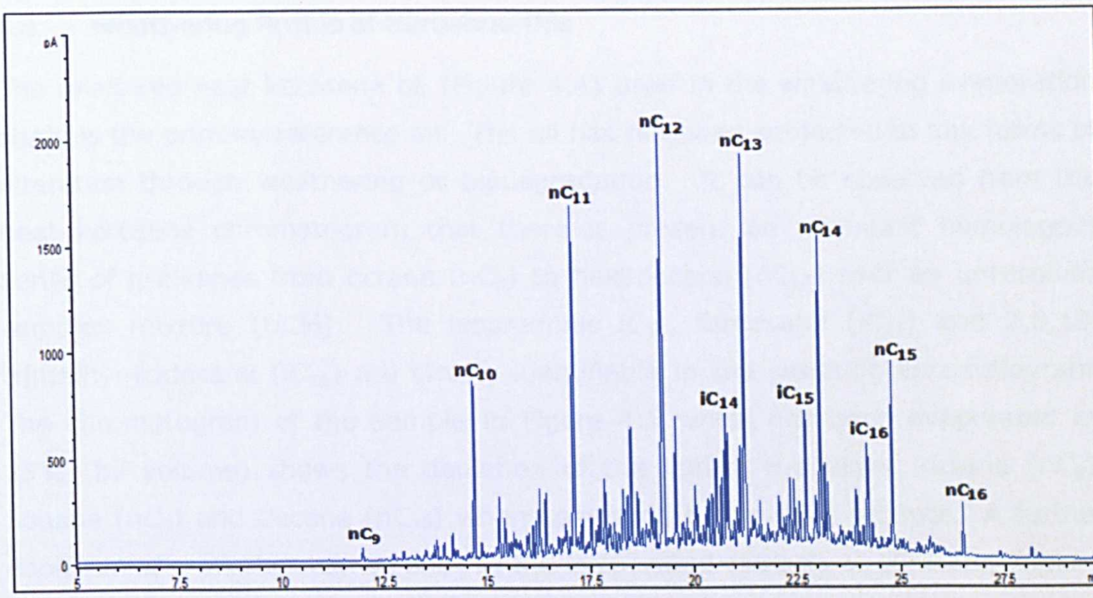


Figure 4.6 Evaporated Kerosene Oil Reduced by 50% (By Volume)

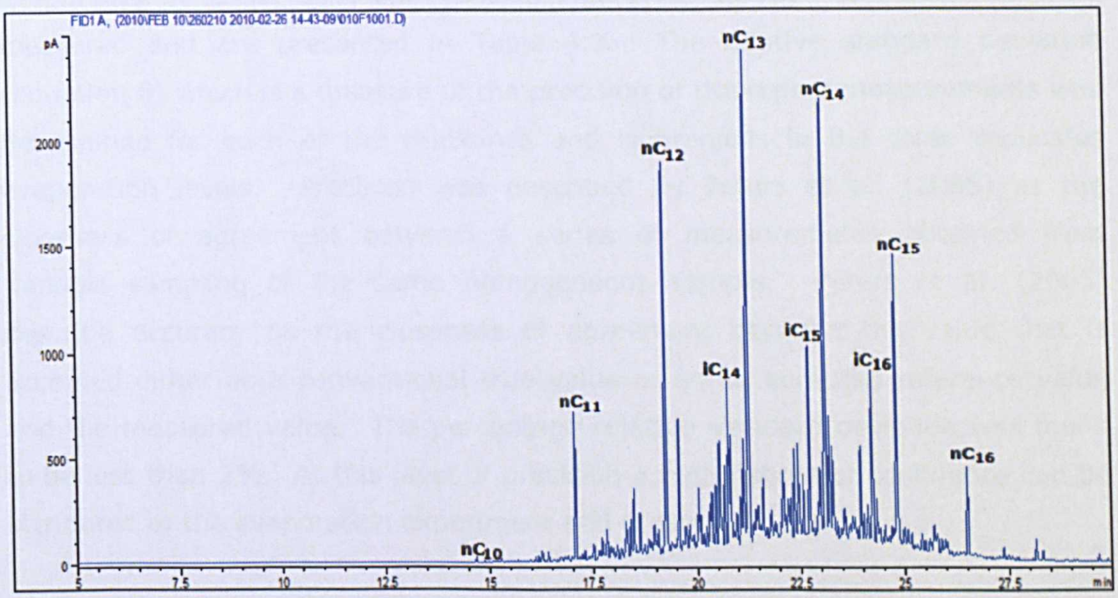


Figure 4.7 Evaporated Kerosene Oil Reduced by 75% (By Volume)

4.3 Weathering Profile of Kerosene Oils

The unaltered neat kerosene oil (Figure 4.4) used in the weathering evaporation study is the primary reference oil. The oil has not been subjected to any forms of alteration through weathering or biodegradation. It can be observed from the neat kerosene chromatogram that there is present an abundant homologous series of n-alkanes from octane (nC_8) to hexadecane (nC_{16}) over an unresolved complex mixture (UCM). The isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) are clearly identifiable in the resulting chromatogram. The chromatogram of the sample in Figure 4.5 which has been evaporated by 25% (by volume) shows the depletion of the lighter n-alkanes, octane (nC_8), nonane (nC_9) and decane (nC_{10}) when compared to the neat product. A further depletion of n-alkanes can be seen in the evaporated extracts at 50% and 75%.

The peak heights of each of the n-alkanes and isoprenoids identified in the evaporation chromatograms from the neat kerosene, and the kerosene evaporated by 25%, 50% and 75% in Figure 4.4 through to Figure 4.7 were measured and are presented in Table 4.1. The relative standard deviation (Equation 6) which is a measure of the precision of the repeat measurements was determined for each of the n-alkanes and isoprenoids in the three replicates evaporation levels. Precision was described by Peters et al. (2005) as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample. Peters et al. (2005) describe accuracy as the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the measured value. The percentage relative standard deviation was found to be less than 2%. At this level of precision a high degree of confidence can be attributed to the evaporation experiment and analysis.

$$\%RSD = \frac{S \times 100}{M}$$

Where S is the total standard deviation and where M is the mean of the results.

Equation 6 Percentage Relative Standard Deviation (%RSD) (EA, 2006)

Table 4.1 Peak Heights of the n-Alkanes and Isoprenoids

Carbon Number	Neat Kerosene (100%)				Evaporated Kerosene by 25%				Evaporated Kerosene by 50%				Evaporated Kerosene by 75%			
	Extract 1	Extract 2	Extract 3	%RSD	Extract 1	Extract 2	Extract 3	%RSD	Extract 1	Extract 2	Extract 3	%RSD	Extract 1	Extract 2	Extract 3	%RSD
nC8	3211733	3215688	3159881	0.97	221650	221192	217720	0.98	0	0	0	0.00	0	0	0	0.00
nC9	10553953	10575261	10566439	0.10	5320572	5324772	5315482	0.09	459952	461392	458884	0.27	0	0	0	0.00
nC10	12823930	12859352	12764020	0.38	11874788	11972110	11820914	0.64	6445806	6459874	6463089	0.14	154539	153736	152653	0.62
nC11	12319338	12269205	12187537	0.54	13680020	13978382	13567370	1.55	12597163	12637686	12555014	0.33	5385270	5378445	5416218	0.37
nC12	11093313	11112617	11024902	0.42	13405903	13520898	13381839	0.55	15120863	15094910	15081003	0.13	14205713	14169010	14202726	0.14
nC13	9480218	9453488	9521863	0.36	11789394	11909589	11879784	0.53	14333298	14357791	14357328	0.10	18073483	18053227	18042914	0.09
nC14	6952434	6949633	6930024	0.18	9009528	8969594	8997217	0.23	11138422	11186746	11129091	0.28	15748049	15757239	15810946	0.22
nC15	3971590	3909770	3990348	1.07	5188209	5213035	5160756	0.50	6886422	6866680	6826252	0.45	10527582	10574478	10531809	0.25
nC16	1052570	1053998	1067920	0.80	1466992	1413587	1421308	2.00	1932731	1938063	1960671	0.76	3266257	3326656	3305275	0.93
Isoprenoid Number	Neat Kerosene (100%)				Evaporated Kerosene by 25%				Evaporated Kerosene by 50%				Evaporated Kerosene by 75%			
	Extract 1	Extract 2	Extract 3	%RSD	Extract 1	Extract 2	Extract 3	%RSD	Extract 1	Extract 2	Extract 3	%RSD	Extract 1	Extract 2	Extract 3	%RSD
iC14	2981536	2920001	2922604	1.18	3850341	3983761	3859675	1.91	4912585	4917582	4913868	0.05	5982084	6012985	6003301	0.26
iC15	2944142	2923551	2950178	0.47	3818818	3823813	3885106	0.96	4875206	4903696	4897567	0.31	6943454	6985642	7006012	0.46
iC16	2023877	2028561	2023981	0.13	2795935	2800018	2841687	0.90	3802645	3827147	3819723	0.33	6314070	6300194	6301090	0.12

In order to demonstrate the evaporation profile, the average of the data at each level of evaporation rates were calculated and presented in Table 4.2. The neat kerosene oil was used as the reference oil and all of the n-alkane peak areas were normalised to the total of the n-alkane peak area (Table 4.2). The data was plotted on a histogram which showed a classic distribution pattern associated with the kerosene. The histogram associated with the evaporated oil sample indicated losses of the lighter n-alkanes (Figure 4.8). Figure 4.9 illustrates the distribution of isoprenoids.

Table 4.2 Normalised Evaporation Data to Total n-Alkane and Isoprenoids

n-Alkane	Average Measured in Neat Kerosene	Average Measured in Kerosene Evaporated by 25%	Average Measured in Kerosene Evaporated by 50%	Average Measured in Kerosene Evaporated by 75%
nC_8	3195767	220187	0	0
nC_9	10565218	5320275	460076	0
nC_{10}	12815767	11889271	6456256	153643
nC_{11}	12258693	13741924	12596621	5393311
nC_{12}	11076944	13436213	15098925	14192483
nC_{13}	9485190	11859589	14349472	18056541
nC_{14}	6944030	8992113	11151420	15772078
nC_{15}	3957236	5187333	6859785	10544623
nC_{16}	1058163	1433962	1943822	3299396
Total nC_8 - nC_{16}	**71357008**	**72080867**	**68916377**	**67412075**
n-Alkane	**Normalised % Average Kerosene (Neat)**	**Normalised % Average Kerosene (Evaporated by 25%)**	**Normalised % Average Kerosene (Evaporated by 50%)**	**Normalised % Average Kerosene (Evaporated by 75%)**
nC_8	4.48	0.31	0.00	0.00
nC_9	14.81	7.38	0.67	0.00
nC_{10}	17.96	16.49	9.37	0.23
nC_{11}	17.18	19.06	18.28	8.00
nC_{12}	15.52	18.64	21.91	21.05
nC_{13}	13.29	16.45	20.82	26.79
nC_{14}	9.73	12.48	16.18	23.40
nC_{15}	5.55	7.20	9.95	15.64
nC_{16}	1.48	1.99	2.82	4.89
Total nC_8 - nC_{16}	**100%**	**100%**	**100%**	**100%**

Isoprenoid	Average Kerosene (Neat)	Average Kerosene (Evaporated by 25%)	Average Kerosene (Evaporated by 50%)	Average Kerosene (Evaporated by 75%)
iC14	2941380	3897926	4914678	5999457
iC15	2939290	3842579	4892156	6978369
iC16	2025473	2812547	3816505	6305118
Total iC14 – iC16	**7906144**	**10553051**	**13623340**	**19282944**
Carbon Number	**Normalised % Average Kerosene (Neat)**	**Normalised % Average Kerosene (Evaporated by 25%)**	**Normalised % Average Kerosene (Evaporated by 50%)**	**Normalised % Average Kerosene (Evaporated by 75%)**
iC14	37	37	36	31
iC15	37	36	36	36
iC16	26	27	28	33
Total nC_8 - nC_{16}	**100%**	**100%**	**100%**	**100%**

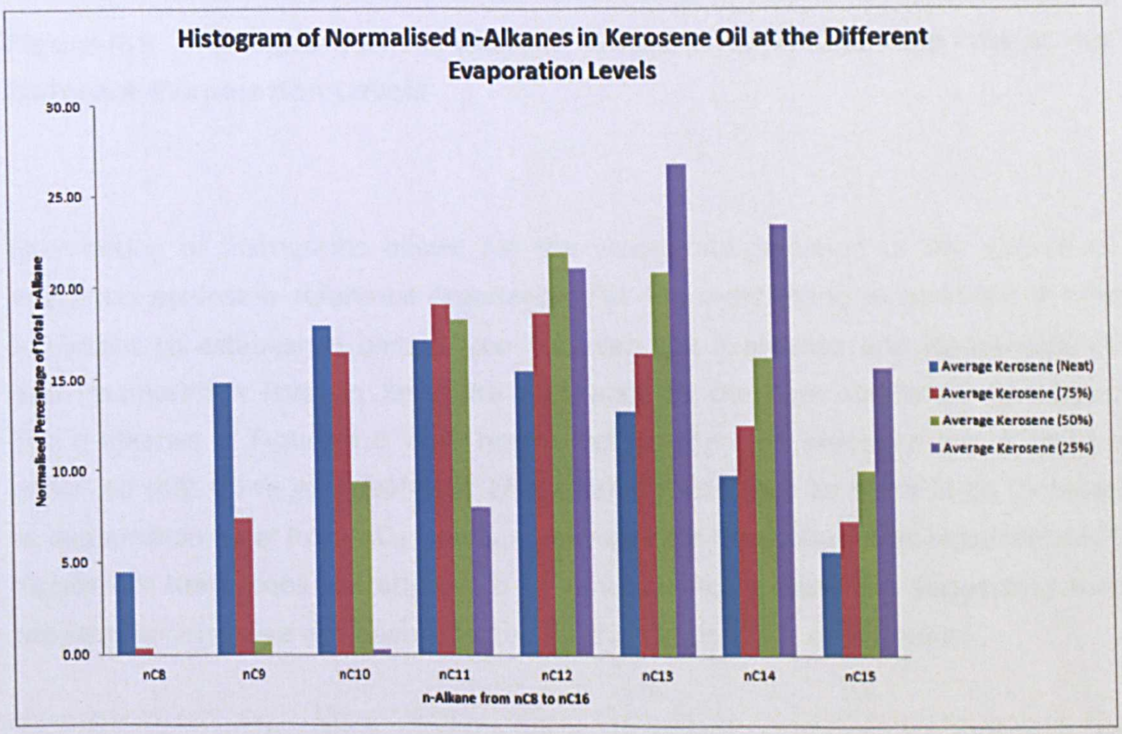


Figure 4.8 Histogram of Normalised n-Alkanes in Kerosene Oils at the Different Evaporation Levels

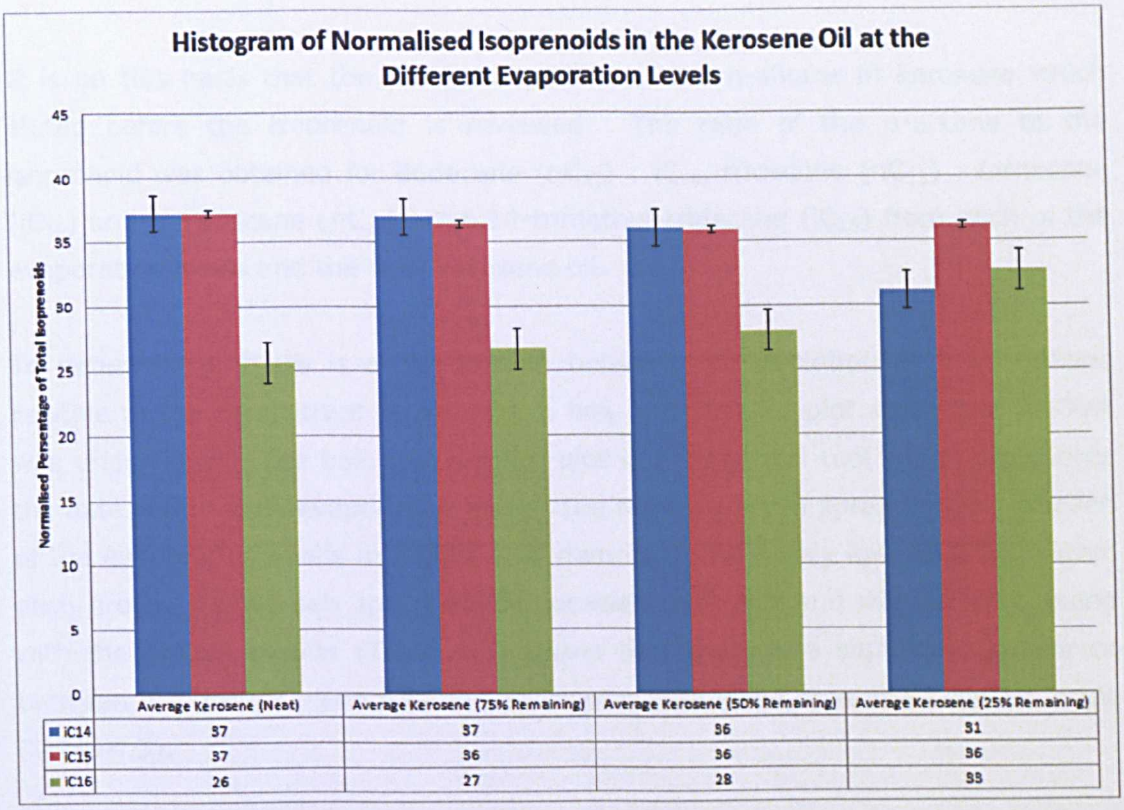


Figure 4.9 Histogram of Normalised Isoprenoids in Kerosene Oils at the Different Evaporation Levels

Normalising of histograms allows for the visual interpretation of the degree of alteration against a reference standard. For the weathering experiment it was important to establish a comparison between the n-alkanes and isoprenoids at each evaporation level in kerosene compared to the neat unaltered kerosene. The n-alkanes in Figure 4.8 were normalised to the neat kerosene and it can be observed that there is a depletion of n-alkane abundance as there is an increase in evaporation level from nC_8 to nC_{12}. However for the isoprenoids represented in Figure 4.9 there does not appear to be any significant depletion suggesting that the isoprenoids have not been affected by the evaporation experiment.

4.4 Correlation of n-Alkane Depletion Over Isoprenoid

The disparity in the rate of biodegradation among hydrocarbon classes is due to the isoprenoids degrading more slowly than comparable n-alkanes (Stout et al., 2002a). This forms the basis for the method described by Christensen and Larsen (1993), allowing for the use of the heptadecane (nC_{17}) : pristane ratio to be used to date middle distillates.

It is on this basis that the rate of depletion of the n-alkane in kerosene which eluted before the isoprenoid is reviewed. The ratio of the n-alkane to the isoprenoid was obtained for dodecane (nC_{12}) : iC_{14}, tridecane (nC_{13}) : farnesane (iC_{15}) and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) from each of the evaporation levels and the neat kerosene oil.

To determine if there is a relationship between the depletion of the n-alkane relative to the recalcitrant isoprenoid, a box and whisker plot study and ANOVA was undertaken. The box and whisker plot is a statistical tool which represents the data within each evaporation level. The tight observed spread shown at each of the evaporation levels in Figure 4.10 demonstrates a very low deviation within each group. Conversely the distance between each box and whisker plot, along with the ANOVA results (Table 4.3) shows that there is a significant difference between the groups. The f-value was significantly lower than the f-critical value (Table 4.3).

The linear regression plot for each of the n-alkane to isoprenoid demonstrated a linear relationship with coefficient of determination (R^2) values (Table 4.3) of:

- Dodecane (nC_{12}) : iC_{14} versus Percentage Evaporated $R^2 = 0.9808$;
- Tridecane (nC_{13}) : Farnesane (iC_{15}) versus Percentage Evaporated $R^2 = 0.9729$; and
- Tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus Percentage Evaporated $R^2 = 0.9900$

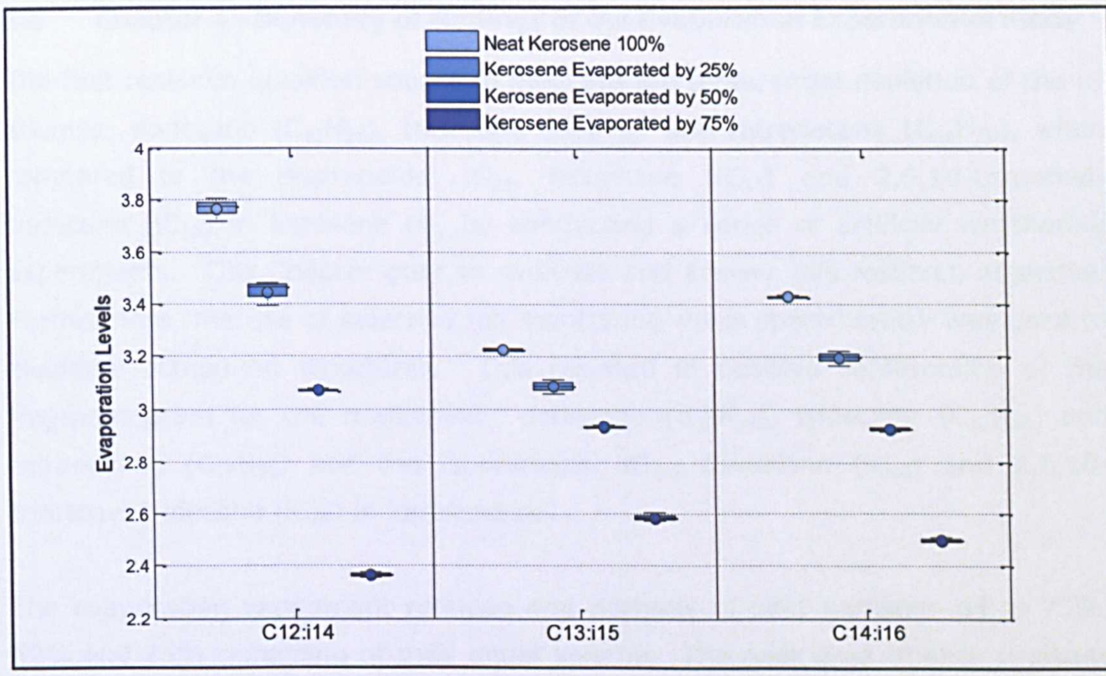


Figure 4.10 Box and Whisker Plot in Kerosene Oils at the Different Evaporation Levels for Each of the Diagnostic Ratios

Table 4.3 ANOVA Results For the Evaporation Study

Ratio	Source of Variance	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
nC_{12} : iC_{14}	**Between Groups**	16.5918	1	16.5918	19.9738	0.00019	4.30095
	Within Groups	18.275	22	0.83068			
	Total	34.8668	23				
	Source of Variance	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
nC_{13} : iC_{15}	**Between Groups**	12.7646	1	12.7646	17.9067	0.00034	4.30095
	Within Groups	15.6824	22	0.71284			
	Total	28.447	23				
	Source of Variance	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
nC_{14} : iC_{16}	**Between Groups**	13.7216	1	13.7216	18.372	0.0003	4.30095
	Within Groups	16.4313	22	0.74688			
	Total	30.1528	23				

4.5 Chapter 4 - Summary of Findings of the Evaporation Experimental Study

The first research question sought to evaluate the preferential depletion of the n-alkanes: dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$), when compared to the isoprenoids: iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in kerosene oil, by conducting a series of artificial weathering experiments. This Chapter goes to evaluate and answer this research objective. Furthermore, the use of selective ion monitoring mass spectrometry was used to elucidate compound structures. This resulted in positive confirmation of the fragmentogram for the n-alkanes: dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) and the isoprenoids: iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in kerosene oil.

The evaporation experiment reduced 4ml portions of neat kerosene oil to 75%, 50% and 25% remaining of their initial volume. The peak area of each n-alkane and isoprenoid was measured at each evaporation level in triplicate and the RSD of 2% was calculated. A box and whisker plot of the ratios against number of days from release showed a very low distribution within each evaporation level, supporting the observed RSD of 2%. Conversely the ANOVA evaluation demonstrated a significant difference between the evaporation levels which is to be expected as the evaporation levels change the n-alkanes are appearing to deplete preferentially.

5.0 Chapter 5 Results

The research work presented in this chapter focuses on a detailed investigation into 6,100 chromatograms. These chromatograms were derived from the extraction of soil samples submitted by environmental consultants and regulators to Jones Environmental Forensics Laboratory (JEFL) between 2009 and 2011. Of the 6,100 chromatograms reviewed, 473 chromatograms had profiles which were characteristic of a kerosene home heating oil contaminant and these were then included and evaluated in this research. These 473 samples were submitted to the laboratory by nine different environmental consultants firms. A number of samples were deemed inadmissible as described in Chapter 3. The research is based on the detailed analysis of the remaining samples. The identities of the consultants were anonymised in the dissertation because of the laboratory's standard confidentiality agreements. Confidentiality was maintained by generating a unique identification number which was given to each consultant. The samples from consultants 1 and 4 were primarily used within the study. Section 3.14.1 of Chapter 3, describes the process involved in the selection of these consultants and samples.

5.1 Age Estimation Dataset Construction

The selection of the most appropriate n-alkanes (dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$)) and isoprenoids (iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16})) for the model was described in Chapter 4 in the analysis of kerosene and weathering experiment. The integration procedure used for the gathering of the n-alkane and isoprenoid peak heights is described in Appendix 1. For each of the 473 samples, the peak height of the n-alkane to the peak height of the isoprenoid was calculated for each of three pairs of n-alkanes and isoprenoids:

- Dodecane (nC_{12}) : iC_{14};
- Tridecane (nC_{13}) : Farnesane (iC_{15}); and
- Tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}).

These ratios were based on the preferential depletion of the n-alkane in preference to the isoprenoid. The ratio of the peak height of the n-alkane to the isoprenoid tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) was calculated and reported in Table 5.3. The relevant sample site data including the environmental consultant firm, project manager's name, consultant's project name, soil sample site identification, site location, soil sample depth, reported extractable petroleum hydrocarbon concentration (mg/kg) and sampling date were obtained from a review of the certificate of analysis report produced by JEFL for each consultant. Each of the environmental consultants was contacted and where possible the suspected home heating oil release date was obtained. If a release date was not known, or if a continuous release was occurring at the spill site, then those samples were deemed ineligible for inclusion. As described in section 3.14.2 of Chapter 3, samples were subject to these and other criteria for eligibility. This resulted in a final total of 43 samples, which are used in the model and the statistical assessment described in this chapter.

5.1.1 Minimum Depth and Concentration

As described in the Chapter 2 Literature Review, the Christensen and Larsen (1993) study on diesel suggested that the assessment of age may be valid only if a number of conditions were met - this included a minimum depth below the surface and a minimum concentration of the extractable petroleum hydrocarbon (EPH). To establish the minimum depth and concentration to be used for the model the ratio of tetradecane ($C_{14}H_{30}$) to 2,6,10-trimethyl-tridecane (iC_{16}) versus the known age of release of kerosene was plotted from the complete dataset (Appendix 3). Samples were omitted from this assessment if any of the factors describe in paragraph 3.14.2 above were found. A linear regression trend line was calculated for the data and the coefficient of determination (R^2) was used as a means of determining the point at which the minimum depth and concentration was achieved to produce a R^2 of greater than 0.7. A conservative cut point for R^2 of 0.7 or higher was recommended by Wiens, Dale and Boyce et al. (2008).

To determine appropriate values for the minimum depth threshold (DTm) and for the minimum concentration threshold (CTm), the selected nC_{14} : iC_{16} ratio was plotted against known age of release, for varying values of DTm and CTm – using the following procedure.

- Iteratively adjusting DTm and CTm – and disregarding samples not meeting either threshold, plot the nC_{14} : IC_{16} ratio against the age of release for all values in the remaining dataset, and record the linearity (R^2) for the plotted trend line.
- This procedure is repeated for combinations of varying DTm and CTm, to produce a matrix showing the linearity (R^2 of nC_{14} : IC_{16} Vs age of release) for each combination of DTm and CTm.

This calculation was performed for 12 different values of CTm (ranging from 0mg/kg to 1,400mg/kg), and for 14 different values of DTm (ranging from 0m to 0.75m). The matrix of the resulting 168 R^2 values and p-values is shown in Table 5.1 and Table 5.2. Applying the conservative cut point for R^2 of 0.7 or higher as recommended by Wiens, Dale and Boyce et al. (2008), The following minimum values are apparent.

- The appropriate minimum depth threshold is 0.4m.
- The appropriate minimum concentration threshold is 1,000mg/kg.

Probabilities were assessed from the regression analysis where the null hypothesis was established as been that there was no correlation between the ratio of tetradecane ($C_{14}H_{30}$) to 2,6,10-trimethyl-tridecane (IC_{16}) versus the known age of release of kerosene when a linear regression line was plotted. Using both calculated R^2 values and p-values it was observed that small changes observed in the p-values appeared to increase as both depth and concentration increased. As the p-value moves towards very small value 3.49E-14 at 0.4m and 1,000 mg/kg this provides for a higher degree of confidence to disprove the null hypothesis of no correlation. This is further supported by any increase in R^2 value 0.7200 of greater than 0.7 as the p-value reduces significantly (Wiens, Dale and Boyce et al., 2008; Marques de Sa, 2007)

Once these minimum thresholds were applied, the dataset was reduced to 43 qualifying samples.

Table 5.1 Determination of Minimum Sample Depth and Concentration from the Ratio of Tetradecane ($C_{14}H_{30}$) to 2,6,10-Trimethyl-tridecane (iC_{16}) versus the Known Age of Release of Kerosene Using R^2.

	R^2 Value at Different Depth in Meters and at Different Concentrations													
mg/kg	**<0 (m)**	**0.1 (m)**	**0.2 (m)**	**0.25 (m)**	**0.3 (m)**	**0.35 (m)**	**0.4 (m)**	**0.45 (m)**	**0.5 (m)**	**0.55 (m)**	**0.6 (m)**	**0.65 (m)**	**0.7 (m)**	**0.75 (m)**
<0 mg/kg	0.0089	0.0097	0.0132	0.0173	0.02	0.0507	0.0751	0.0536	0.0518	0.0102	0.016	0.018	0.0107	0.0015
100 mg/kg	0.0058	0.0064	0.0088	0.009	0.0093	0.0348	0.0572	0.0301	0.0288	0.0096	0.0156	0.016	0.0092	0.0023
200 mg/kg	0.0086	0.0094	0.0128	0.0138	0.0109	0.0459	0.0761	0.0439	0.0426	0.0418	0.0432	0.032	0.0169	0.0028
300 mg/kg	0.0162	0.0176	0.0233	0.0277	0.0237	0.0639	0.0822	0.0483	0.047	0.0513	0.0457	0.035	0.0178	0.0035
400 mg/kg	0.0232	0.0249	0.032	0.0378	0.0315	0.0716	0.0947	0.066	0.0648	0.0883	0.0881	0.076	0.0442	0.0159
500 mg/kg	0.0252	0.0272	0.0355	0.0499	0.0312	0.0775	0.1075	0.09	0.0887	0.1075	0.1069	0.094	0.0597	0.0426
600 mg/kg	0.0022	0.0021	0.0007	0.0137	0.0186	0.0632	0.101	0.086	0.085	0.1607	0.1103	0.1	0.0573	0.0426
700 mg/kg	0.0044	0.0038	0.0004	0.0108	0.0148	0.0625	0.0722	0.0612	0.0607	0.1154	0.1103	0.1	0.0573	0.0426
800 mg/k	0.0075	0.0059	0.0003	0.0018	0.0026	0.0236	0.0358	0.0291	0.0291	0.0204	0.0191	0.017	0.0147	0.0089
1000 mg/kg	0.0099	0.0081	3.00E-05	0.0024	0.0038	0.093	**0.7200**	**0.8065**	**0.8128**	**0.971**	**0.9709**	**0.979**	**0.9793**	**0.9903**
1200 mg/kg	0.009	0.0073	8.00E-05	0.0021	0.0033	0.0892	**0.7728**	**0.9052**	**0.9161**	**0.9715**	**0.9714**	**0.98**	**0.9795**	**0.9924**
1400 mg/kg	0.0417	0.0372	0.0491	0.0413	0.0176	0.0402	**0.7381**	**0.7325**	**0.6832**	**0.7393**	**0.7396**	**0.793**	**0.7903**	**0.8058**

Table 5.2 Determination of Minimum Sample Depth and Concentration from the Ratio of Tetradecane ($C_{14}H_{30}$) to 2,6,10-Trimethyl-tridecane (iC_{16}) versus the Known Age of Release of Kerosene Using p-Values.

	p-Value at Different Depth in Meters and at Different Concentrations													
mg/kg	**<0 (m)**	**0.1 (m)**	**0.2 (m)**	**0.25 (m)**	**0.3 (m)**	**0.35 (m)**	**0.4 (m)**	**0.45 (m)**	**0.5 (m)**	**0.55 (m)**	**0.6 (m)**	**0.65 (m)**	**0.7 (m)**	**0.75 (m)**
<0 mg/kg	0.0186	0.0209	0.0142	0.0069	0.0055	0.0072	0.0341	0.0246	0.0269	0.0094	0.0104	0.0495	0.0509	0.0701
100 mg/kg	0.0066	0.0074	0.0040	0.0011	0.0007	0.0010	0.0059	0.0049	0.0039	0.0049	0.0061	0.0303	0.0268	0.0221
200 mg/kg	0.0094	0.0102	0.0036	0.0004	0.0001	0.0002	0.0019	0.0025	0.0021	0.0028	0.0028	0.0114	0.0107	0.0082
300 mg/kg	0.0366	0.0384	0.0168	0.0022	0.0008	0.0013	0.0013	0.0368	0.0015	0.0028	0.0028	0.0092	0.0092	0.0071
400 mg/kg	0.1267	0.1300	0.0671	0.0114	0.0041	0.0055	0.0054	0.0732	0.0088	0.0173	0.0173	0.0755	0.0732	0.0615
500 mg/kg	0.1654	0.1698	0.0974	0.0182	0.0064	0.0078	0.0076	0.0836	0.0090	0.0152	0.0153	0.0685	0.0652	0.0547
600 mg/kg	0.0143	0.0143	0.0010	0.0022	0.0026	0.0032	0.0032	0.1046	0.0046	0.0081	0.0081	0.0218	0.0232	0.0169
700 mg/kg	0.0729	0.0691	0.0010	0.0015	0.0018	0.0023	0.0021	0.0771	0.0058	0.0175	0.0175	0.0218	0.0232	0.0169
800 mg/k	0.0193	0.0123	0.0236	0.0027	0.0021	0.0025	0.0019	0.0125	0.0031	0.0228	0.0128	0.0001	0.0001	0.0011
1000 mg/kg	0.5017	0.4764	0.4310	0.4076	0.3015	0.2012	**3.49E-13**	**2.79E-13**	**1.67E-13**	**6.83E-14**	**2.12E-14**	**1.29E-13**	**2.92E-09**	**6.48E-05**
1200 mg/kg	0.5608	0.5359	0.5311	0.3026	0.4014	0.4022	**2.48E-11**	**6.52E-12**	**3.48E-12**	**1.36E-12**	**4.68E-13**	**3.33E-08**	**1.03E-07**	**1.32E-04**
1400 mg/kg	0.5845	0.7024	0.7917	0.6844	0.7042	0.7115	**2.56E-11**	**5.51E-12**	**4.18E-13**	**1.54E-12**	**5.37E-12**	**4.13E-08**	**1.10E-09**	**2.21E-05**

The n-alkane to isoprenoid ratio with the estimated number of days following release for the 43 samples used in the generation of the age estimation model is presented in Table 5.3. This table consists of the following information.

- The sample number is given for the chromatogram examined out of the original 473 samples;
- The consultant identification number is given. After all the constraints in 3.14.2 were applied, all the remaining eligible samples came from consultants 1 and 4;
- The depth at which this sample was taken by the field consultant is given. A minimum depth of 0.4m was selected. Chapter 3, provides the rational behind the selection of this minimum depth. Soil depth ranged from 0.4m to 1.9m with 58% of the soils between 0.4m and 0.5m, 42% of the soils between 0.6m and 1.9m;
- The concentration in mg/kg which was analytically determined using JEFL extractable petroleum hydrocarbon (EPH) analysis method is given. In all cases the concentration is dominated by kerosene. A minimum concentration of 1,000 mg/kg was selected. Chapter 3 provides the rationale behind the selection of this minimum concentration. EPH concentration ranged from 1,025 mg/kg to 18,734 mg/kg with the 77% of the samples between 1,000 mg/kg – 7,000mg/kg with the remaining 23% between 7,001 mg/kg – 19,000 mg/kg;
- The ratio is given of n-alkane to isoprenoids, tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) for the selected compounds. The rational for the selection of these ratios was established in Chapter 4 following the analysis of kerosene and the weathering experiments. The selection of the diagnostic ratios is described in Chapter 3. The ratios ranged from 0.15 on day one and to 0.49 on day 35;
- The sampling date is given. This was obtained from the chain of custody reported in the certificate of analysis for each sample by the environmental consultant when they were submitting the samples for analysis to JEFL;
- The release date was obtained following a series of communications with the individual consultant from each of the selected firms; and
- The known number of days following release represents the difference in days between the suspected oil release date and the sampling date.

Table 5.3 n-Alkane and Isoprenoid Database Consultants 1 and 4

Sample Number	Consultant Number	Depth (m)	Conc. (mg/kg)	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date	Known Days
18	Consultant 4	0.75	14928	5.27	5.46	6.44	28/09/2009	29/09/2009	1
201	Consultant 4	0.5	18734	6.04	5.97	6.55	29/10/2009	30/10/2009	1
202	Consultant 4	0.75	9233	6.59	5.60	6.13	29/10/2009	30/10/2009	1
242	Consultant 4	0.4	6718	4.43	5.48	5.76	14/10/2009	16/10/2009	2
243	Consultant 4	0.4	2725	4.59	5.53	5.54	14/10/2009	16/10/2009	2
244	Consultant 4	0.5	1684	4.47	5.57	6.18	14/10/2009	16/10/2009	2
245	Consultant 4	0.5	5442	4.37	4.95	5.21	14/10/2009	16/10/2009	2
193	Consultant 4	0.5	1848	3.45	5.02	4.47	24/10/2009	27/10/2009	3
344	Consultant 4	0.7	2568	3.94	4.25	4.88	24/11/2009	27/11/2009	3
345	Consultant 4	0.4	7823	3.23	4.97	4.43	24/11/2009	27/11/2009	3
346	Consultant 4	0.8	5119	4.13	4.30	4.15	24/11/2009	27/11/2009	3
352	Consultant 4	1	1025	6.07	4.57	4.44	26/11/2009	30/11/2009	4
464	Consultant 4	1	5269	3.53	6.16	4.75	10/12/2009	14/12/2009	4
466	Consultant 4	1	2177	3.61	5.03	4.73	10/12/2009	14/12/2009	4
467	Consultant 4	0.4	4497	2.91	4.40	4.47	10/12/2009	14/12/2009	4
50	Consultant 4	0.9	1117	5.44	5.62	4.99	01/10/2009	06/10/2009	5
51	Consultant 4	0.5	1752	4.40	5.44	4.86	01/10/2009	06/10/2009	5
338	Consultant 4	0.4	4841	3.22	3.74	4.00	08/11/2009	13/11/2009	5
339	Consultant 4	0.8	3668	3.87	4.68	4.39	08/11/2009	13/11/2009	5
435	Consultant 4	0.5	2724	3.56	5.40	4.82	28/11/2009	03/12/2009	5
437	Consultant 4	0.5	1445	4.69	5.68	4.62	29/11/2009	04/12/2009	5
38	Consultant 4	0.6	6216	5.51	4.95	4.55	19/09/2009	25/09/2009	6
39	Consultant 4	0.4	4644	5.09	4.43	4.50	19/09/2009	25/09/2009	6
35	Consultant 4	0.4	3354	3.97	4.30	4.20	20/09/2009	29/09/2009	9
36	Consultant 4	0.4	9324	4.15	4.41	4.40	20/09/2009	29/09/2009	9
438	Consultant 4	0.4	2330	3.52	3.99	3.24	29/11/2009	09/12/2009	10
439	Consultant 4	0.65	10481	4.28	4.64	3.72	01/12/2009	11/12/2009	10
440	Consultant 4	0.65	4578	3.40	3.93	3.29	01/12/2009	11/12/2009	10
240	Consultant 4	1	1614	3.88	4.37	3.01	18/10/2009	30/10/2009	12
46	Consultant 4	2.5	16615	2.30	3.05	3.03	16/09/2009	01/10/2009	15
77	Consultant 1	0.4	8268	1.26	3.77	3.62	22/09/2009	07/10/2009	15
78	Consultant 1	1.60-1.90	12492	3.12	3.38	3.30	22/09/2009	07/10/2009	15
453	Consultant 4	0.5	6315	2.94	4.20	2.88	13/08/2010	31/08/2010	18
275	Consultant 1	0.90-0.10	13734	2.84	2.33	2.65	16/09/2009	05/10/2009	19
96	Consultant 1	0.4	2432	3.40	3.83	2.98	14/10/2008	03/11/2008	20
472	Consultant 4	0.45	4301	4.75	3.82	2.55	23/10/2010	15/11/2010	23
256	Consultant 1	0.4	2955	4.95	3.83	2.68	10/10/2009	03/11/2009	24
107	Consultant 4	0.4	2733	2.99	1.96	2.76	01/09/2009	25/09/2009	24
387	Consultant 1	0.60-0.70	4512	1.79	1.90	2.18	01/11/2009	30/11/2009	29

Sample Number	Consultant Number	Depth (m)	Conc. (mg/kg)	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date	Known Days
55	Consultant 1	1.00-1.50	6630	1.15	1.27	2.48	15/08/2009	14/09/2009	30
258	Consultant 1	0.4	2969	3.20	3.99	2.65	10/10/2009	10/11/2009	31
452	Consultant 4	0.4	1227	0.83	1.75	2.13	20/06/2010	25/07/2010	35
473	Consultant 4	0.5	5910	2.70	2.63	2.35	21/10/2009	01/12/2009	40

5.2 Environmental Factors Influencing the Age Estimation Model

Laboratory methodology has reached a point where analytical error contributes only a very small portion of the total variance seen in data. The USEPA (1992) reported the observation from a site investigation, that 92% of the total variation came from the location of the sample, while only 8% was introduced after the samples were taken, and less than 1% of the total variation could be attributed to the analytical process. One of the most important considerations involving environmental analytical data is whether the samples received from environmental consultants adequately represent the site being investigated. The purpose of environmental sampling and analysis is to obtain a small but informative portion of the sampling site media being investigated. In most site investigations, there is no hard upper limit to the number of soil samples that could be taken, other than the cost of taking the sample. It is essential that the samples selected for analysis accurately characterise the conditions at the site. In general these representative soil samples are analysed, and conclusions about the entire site are made, based on the data obtained from the laboratory.

At home heating oil spills the pollutant is not usually distributed evenly within a site therefore it is important that consideration is given to where and how a sample is taken. One of the largest contributors to variability of analytical data can be attributed to the uneven distribution of the contaminant in the soil (IAEA, 2004; Jones, 2011). The overall quality of the data obtained from the analysis of soil samples can be influenced by a number of variables related to conditions at the site, the sampling design, approach, and the techniques for collection and preparation of the samples in the field. The data presented in Table 5.3 were obtained from soil samples submitted by two separate environmental consultant firms (Consultants 1 and 4). Environmental consultants and field personnel are very competent and suitably qualified, however different firms may have different sampling protocols. In order to evaluate the possible effects of onsite sampling protocols utilized by consultants 1 and 4, consultant 1 was removed from the model and the trend lines were re-fitted to the known number of days versus the ratio of nC_{14} / iC_{16}. It was found that there in general the R^2 value reduced with the removal of the second consultant (Table 5.4). Section 3.14.1 of the methodology Chapter 3, describes the evaluation of the two consultant firms.

Table 5.4 Coefficients of Determination (R^2) with Consultant 1 excluded from the Dataset

Model Type	R^2 Value For Both Consultants	R^2 Value For Consultant 4 Only
Exponential	0.9187	0.8977
Linear	0.7438	0.6760
Power	0.9116	0.8955
Polynomial	0.8952	0.8728
Logarithmic	0.8354	0.7860

5.2.1 The Soil Type

The fate of petroleum products released into the soil will be particularly varied because they consist of complex mixtures of hydrocarbons with greatly differing vapor pressures and water solubilities (Fine, Graber and Yaron, 1996). The physical and chemical characteristics of the soil type found at the sampling locations can influence the retention, transformation and movement of pollutants through the soil (USEPA, 1992). The abiotic processes occurring within porous soil media control the kerosene's distribution and include sorption, volatilization, transformation and transport (Fine et al., 1996). Once kerosene has been released into the soil its fate can be affected by the dynamics of moisture conditions in the subsurface as a result of rainfall, irrigation, and fluctuation of the ground water level (Dror, Gerstl, Prost et al., 2002). However under abiotic conditions Galin, Gerstl and Yaron (1990) found that the stability of kerosene in soil is primarily affected by volatilization. They found this in a laboratory based experiment, reduction of the total concentration in a variety of different soil type mixes (dune sand, loamy sand and silty loam) over a period of 50 days. Acher, Boderie and Yaron (1989) also found that volatilization was the primary factor following release and migration through soil of kerosene based on a review of a sandy clay type soil. Jarsjo, Destouni and Yaron (1994) found that hydrocarbons in the nonane (nC_9) to undecane (nC_{11}) range can be selectively removed through temperature and volatilization processes. Tetradecane (nC_{14}) to pentadecane (nC_{15}) components were characterised by negative selection. Section 3.15.1 of the methodology chapter 3, describes the selection of the isoprenoid ratio tetradecane (nC_{14}) to 2,6,10-trimethyl-tridecane (iC_{16}) which is used in the generation of the model within this chapter. Hydrocarbon biodegradation in soil can be limited by many factors, for example microorganism type, nutrients, pH, temperature, moisture, oxygen and soil properties (Molina-Barahona, Rodriguez-Vazquez, Hernandez-Velasco et al., 2004). The results for different percentages of clay soil types found indicated that organic matter content of the soil may affect the selective volatilization. The distribution of soil types and natural moisture content found within the samples used to generate the age estimation model is presented in Figure 5.1 and Table 5.5. The breakdown of soil types described by the environmental consultants for the sample submitted to JEFL was as follows.

- 37% clay;
- 23% sandy gravely clay;
- 23% clay silt;
- 7% made ground fill;
- 5% topsoil; and
- 5% clay with peat.

These samples were collected by the environmental consultants from a variety of domestic home sites throughout the United Kingdom and Ireland.

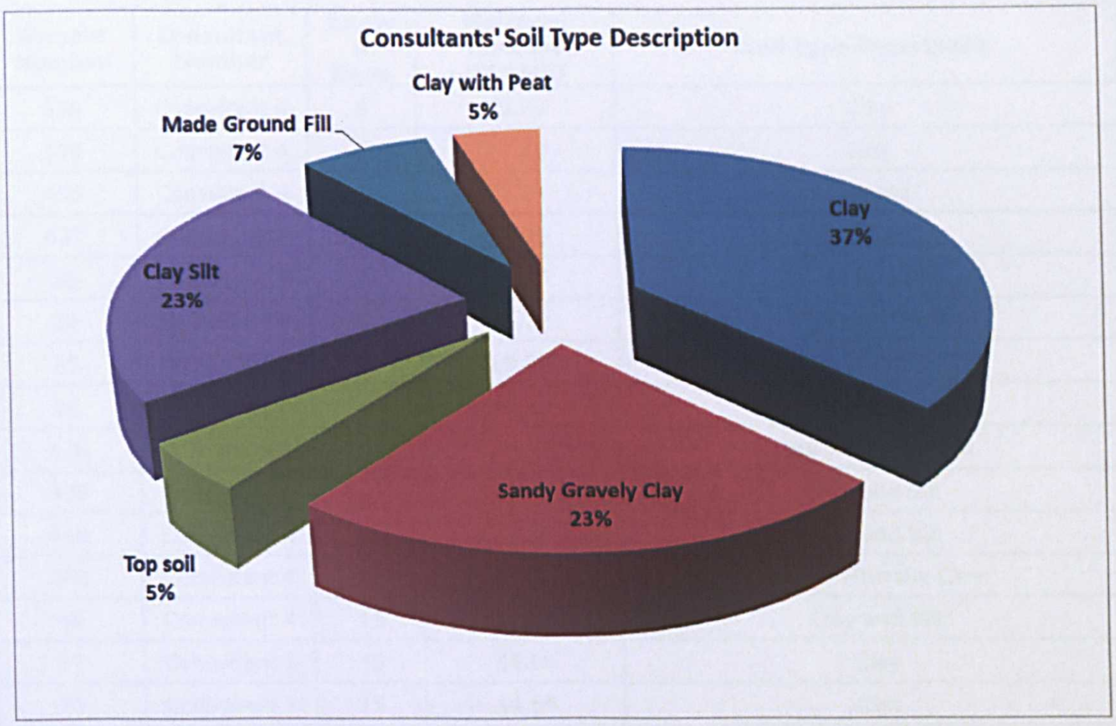


Figure 5.1 Consultants' Soil Type Description

Table 5.5 Soil Type and Percentage Natural Moisture Content

Sample Number	Consultant Number	Known Days	Moisture Content (%NMC)	Soil Type Description
18	Consultant 4	1	24.42	Sandy Gravely Clay
201	Consultant 4	1	21.40	Made Ground Fill
202	Consultant 4	1	22.22	Made Ground Fill
242	Consultant 4	2	36.36	Clay
243	Consultant 4	2	25.64	Clay
244	Consultant 4	2	32.37	Clay
245	Consultant 4	2	30.00	Clay
193	Consultant 4	3	16.86	Dark brown clay topsoil to 0.25m overlying firm red brown sandy clay
344	Consultant 4	3	21.86	Sandy Gravelly Clay
345	Consultant 4	3	21.15	Sandy Gravelly Clay
346	Consultant 4	3	29.29	Sandy Gravelly Clay
352	Consultant 4	4	13.04	Clay
464	Consultant 4	4	20.00	Clay
466	Consultant 4	4	11.94	Clay
467	Consultant 4	4	25.85	Clay
50	Consultant 4	5	24.00	Sandy Gravelly Clay
51	Consultant 4	5	22.00	Sandy Gravelly Clay

Sample Number	Consultant Number	Known Days	Moisture Content (%NMC)	Soil Type Description
338	Consultant 4	5	29.29	Clay
339	Consultant 4	5	17.42	Clay
435	Consultant 4	5	37.01	Top soil
437	Consultant 4	5	25.35	Clay
38	Consultant 4	6	23.13	Clay and Silt
39	Consultant 4	6	12.72	Clay and Silt
35	Consultant 4	9	19.08	Clay with some silt
36	Consultant 4	9	12.83	Clay with some silt
438	Consultant 4	10	10.07	Clay with some silt
439	Consultant 4	10	15.14	Clay and Silt
440	Consultant 4	10	16.89	Clay and Silt
240	Consultant 4	12	49.34	Sandy Gravelly Clay
46	Consultant 4	15	9.25	Clay and Silt
77	Consultant 1	15	56.94	Clay
78	Consultant 1	15	44.55	Clay
453	Consultant 4	18	9.68	topsoil to 0.2m below which was a very sandy material with increasing gravel / clay content below 0.5m
275	Consultant 1	19	55.65	Sandy Gravelly Clay
96	Consultant 1	20	14.43	Sandy Gravelly Clay
472	Consultant 4	23	21.67	topsoil to 0.25m, brown silty clay to 0.5m, below 0.5m the soils had greater gravel content
256	Consultant 1	24	71.82	Soft brown clay
107	Consultant 4	24	24.00	Grey / dark brown rich topsoil
387	Consultant 1	29	68.65	Clay with Peat
55	Consultant 1	30	26.92	Made Ground fill
258	Consultant 1	31	66.57	Soft brown clay
452	Consultant 4	35	65.46	Dark brown rich topsoil below which was clay with peat
473	Consultant 4	40	62.35	topsoil to 0.2m, silty clay and stones to 0.35m, light brown very compact silty clay and stones to 0.5m. Not possible to penetrate beyond 0.5m due to compact nature of soils

Baldrian, Merhautova and Petrankova et al. (2010) commented that spatial heterogeneity is one of the characteristic features of the soil environment and that soil microorganism and microbial processes are affected by soil moisture. The percentage natural moisture content for each of the soil categories are:

- Clay ranged from 11.94% to 71.82%;
- Clay and silt from 9.25% to 62.35%;

- Clay with peat ranged from 65.46% to 68.65%;
- Made ground fill from 21.4% to 26.92%;
- Sandy gravely clay from 9.68% to 55.65%; and
- Topsoil ranged from 24% to 37.01%.

5.3 Volatilization / Evaporation

Weathering processes can be generally categorized into two types: abiotic (physical) weathering and biotic (microbial) weathering. The most significant abiotic weathering process is evaporation, especially for lighter n-alkanes. Evaporation in the short term after a spill is the primary weathering process affecting the chemical composition of spilled kerosene (Wang and Stout, 2007; Wang and Fingas, 2006a). Kaplan et al. (1997) found that weathering could be divided into a number of progressive stages as described in Chapters 2 and 4;

- Abundant n-alkanes;
- Light-end n-alkanes removed;
- More than 90% of n-alkanes removed;
- Alkylcyclohexanes and alkylbenzene removed; and
- Isoprenoids reduced.

The n-alkanes from methane to pentadecane (nC_{15}) with boiling points below 270°C will tend to volatilise (Wang and Stout, 2007). Kerosene falls within the range of octane (nC_8) to Hexadecane (nC_{16}), and has a boiling point range of 200°C to 290°C. Fingas (1995) suggested that the rate of loss of n-alkanes will tend to progress linearly or logarithmically with evaporation. Evaporation was noted by Fingas (1995) as the prime physical change mechanism following a release of crude oil or refined petroleum products. In Chapter 4, *Analysis of Kerosene and Weathering Experiment*, it was determined following the controlled evaporation of neat kerosene within the laboratory, that the ratio of the selected n-alkane : Isoprenoid versus the percentage evaporated followed a linear progression. The coefficient of determination R-squared value (R^2) for the tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus percentage evaporation was found to be R^2 = 0.9900 demonstrating a linear relationship. Dror, Gerstl and Yaron (2001) in a review of a field experiment looking at the leaching and natural attenuation of kerosene concluded that the main process controlling the contamination attenuation was volatilization and redistribution with depth.

The diversity of physical and chemical composition of kerosene influences its migration rate and fate within a soil. Since the mixture is continually changing as the product migrates through a spill zone the organic compounds will partition between the different phases and their degradation rates will be affected (Dror et al., 2001). Biodegradation is considered another major mechanism for the removal of spilled kerosene from the environment (Gouda, Omar and Chekroud et al., 2007).

5.4 Biodegradation

Biodegradation of oil is one of the most important processes involved in weathering and the eventual removal of petroleum from the environment, particularly for the non-volatile components of petroleum. Numerous scientific review articles have covered various aspects of this process and the environmental factors that influence the rate of biodegradation (Zhu et al., 2001). Micro-organisms capable of degrading kerosene and other petroleum hydrocarbons are ubiquitous in soil environments. Over 200 species of micro-organisms have been shown to degrade hydrocarbons ranging from methane to compounds of over 40 carbon atoms (Zobell, 1973). Hydrocarbon degrading bacteria are ubiquitous members of the indigenous bacterial community in soil and aquatic environments (Atlas, 1981). Generally in pristine environments, the relative numbers of microorganisms tend to increase by orders of magnitude (exponentially) in hydrocarbon exposed areas (Roberg, 2007). In pristine ecosystems, hydrocarbon degraders may make up less than 0.1% of the microbial community and in oil-polluted environments, they can constitute up to 100% of the viable micro-organisms (Atlas, 1981). As seen with evaporation in paragraph *5.3 Volatilization / Evaporation*, tracking changes due to evaporation utilises compounds with different volatilities or vapour pressures which show some resistance to biodegradation. Snape, Harvey and Ferguson et al. (2005) found that in kerosene or diesel the light acyclic isoprenoids iC_{13}, iC_{14} and farnesane (iC_{15}) evaporate progressively more readily and can be compared with the heavier isoprenoids such as 2,6,10-trimethyl-tridecane (iC_{16}) and pristane. Similarly for a biodegradation mechanism the utilization of ratios of compounds such as the straight chain alkanes which are known to be degraded in preference to the branched isoprenoids, dodecane (nC_{12}) : iC_{13} up to octadecane (nC_{18}) : phytane provide useful indices of biodegradation (Snape et al., 2005). The most dominant mechanism that breaks down the composition of kerosene in soil is biodegradation which is carried out by natural microbial populations (Shabir et al., 2008).

The extent of biodegradation in soil is dependent on the hydrocarbon source, concentration of the total hydrocarbon, and availability of oxygen and nutrients (Shabir et al., 2008). Hydrocarbons in kerosene do not inhibit microbial activity and under aerobic conditions, they can biodegrade significantly as long as sufficient amounts of nutrients are present (Shabir et al., 2008). Shabir et al. (2008) suggested that less is known about the biodegradability of some commercial products such as kerosene. Binazadeh, Karimi and Li (2009) found that the rate of biodegradation of an n-alkane using micro-organisms followed a linear rate of degradation. However, within this study an exponential rate of decay was determined to be the mechanism of degradation. Exponential regression trend line analysis is a nonlinear regression form of regression analysis which is based on linear least squares regression (MathHolt, 2011; Tamhane and Dunlop, 2000). Unlike linear regression, which plots values along a straight line, exponential regression describes a curve by calculating the array of values needed to plot it (Excel, 2011). The exponential function is $y = ae^{bx}$ where b is the exponential growth rate. The equation breaks down to a linear regression function by taking the natural log of both sides $\ln(y) = \ln(ae^{bx})$ this equation further breaks down to $\ln(y) = \ln(a) + bx$ where the natural log of a ($\ln(a)$) is the y intercept and b is the slope m (MathHolt, 2011; Tamhane and Dunlop, 2000).

5.5 Descriptive Statistics

Descriptive statistics is simply the numerical procedure or graphical techniques used to organise and describe the characteristics or factors of a given sample (Fisher and Marshall, 2009). Statistics can be used to illustrate the characteristics of a group of observations (Marshall and Jonker, 2010) however it is important to consider the influence of abiotic or biotic environmental conditions at the spill location in the selection of the most appropriate statistical model. Statistics is primarily concerned with how to summarise and interpret variables (DeCoster, 1998). These variables include volatilization, biodegradation and chemical alterations of the released kerosene following release into soil. The ultimate objective of the statistical trend line analysis in this chapter is to identify and develop a model that will accurately predict *y* as a function of a set of predictor variables *x* taking into account all of the environmental influences (evaporation and biodegradation) at the spill location. Therefore this section focuses on a number of statistical trend lines that can be applied to define the data within this study taking into account the primary mechanisms of alteration – volatilization and biodegradation. The reliability of each of the trend lines was measured by its coefficient of determination R-squared value (R^2). The coefficient of determination is a popular statistic in the analysis of regression models. Several models can be compared by their R^2 values (McKean and Sievers, 1987). The R^2 value is a number between 0 and 1 that reveals how closely the estimated value for the trend line corresponds to the actual data. A trend line is most reliable when its R^2 value is near 1 (StatTrek, 2011; Roy and Sarangi, 2008).

5.5.1 Linear Regression Model

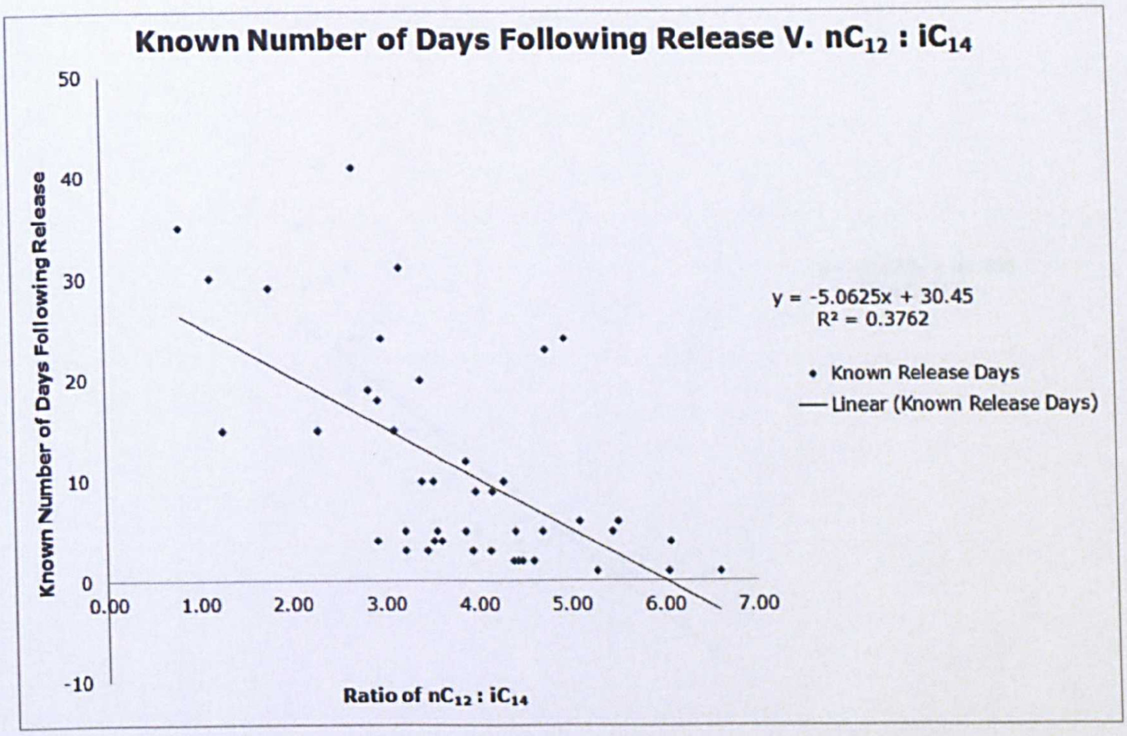
Linear regression is a method that studies relationships between variables, more often to study two variables where one depends upon the other (Rong, 2000), in this case the preferential depletion of the n-alkane over the isoprenoid over the time following the release. The linear regression model trend line allows for the prediction of the result of one variable (time) when given the other variable (n-alkane : isoprenoid ratio). Linear least squares regression is by far the most popular and widely used modeling method (ESH, 2011). The Kaplan, Galperin, Alimi et al., (1996b) equation which was used for diesel releases, used the relationship between heptadecane (nC_{17}) / pristane ratio to simplify the observations of the Christensen and Larsen (1993) followed a linear relationship as did the model presented by Hurst and Schmidt (2005). The two models used the linear equation:

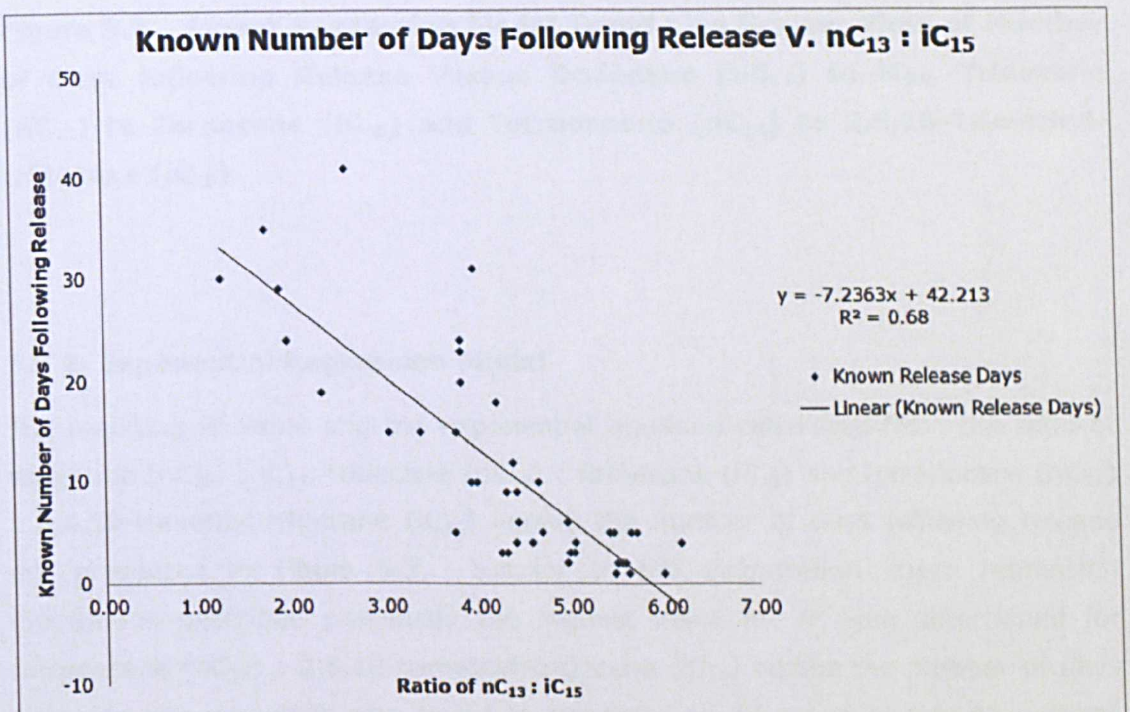
$$y = \beta x + \alpha \text{ or } y = mx + c$$

where *y* is a dependent variable, x is an independent variable, α is the linear intercept, β is the linear slope (Walpole and Myers, 1989; Rong, 2000).

Equation 7 Linear Regression Equation

The resulting R^2 value and the linear equation calculated from the ratios of dodecane (nC_{12}) : iC_{14}, tridecane (nC_{13}) : farnesane (iC_{15}) and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release are presented in Figure 5.2. The highest value for R^2 was determined for tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release was found to generate an R^2 value of 0.7438 with an equation of $y = -7.477x + 41.586$. It can be seen from the linear scatter plots presented in Figure 5.2 that the data appears to have undergone a progressive stage of weathering which was described by Kaplan et al., (1997). This was evident as the lighter n-alkanes and isoprenoids of dodecane (nC_{12}) to tetradecane (nC_{14}) appeared to be subjected to a greater degree of variance after 10 days for plot of number of days versus dodecane (nC_{12}) : iC_{14}, as seen in Figure 5.2, which resulted in a poor R^2 value of 0.3762. Similarly the ratio of number of days versus tridecane (nC_{13}) : farnesane (iC_{15}) demonstrates a significantly scatter of data after 15 days resulting in an R^2 value of 0.68. A conservative cut point for R^2 of 0.7 or higher is recommended by Wiens, Dale and Boyce et al., (2008). This would suggest that the ratio of dodecane (nC_{12}) : iC_{14} and the borderline ratio of tridecane (nC_{13}) : farnesane (iC_{15}) versus number of days is unlikely to produce a strong predictive model of age estimation of kerosene. However the equation generated from the tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release may produce a strong predictive model. This will be evaluated later in this chapter under the model validation section.


Known Number of Days Following Release V. nC_{12} : iC_{14}
Known Number of Days Following Release
Ratio of nC_{12} : iC_{14}
y = -5.0625x + 30.45
R^2 = 0.3762
Known Release Days
Linear (Known Release Days)


Known Number of Days Following Release V. nC_{13} : iC_{15}
Known Number of Days Following Release
Ratio of nC_{13} : iC_{15}
y = -7.2363x + 42.213
R^2 = 0.68
Known Release Days
Linear (Known Release Days)

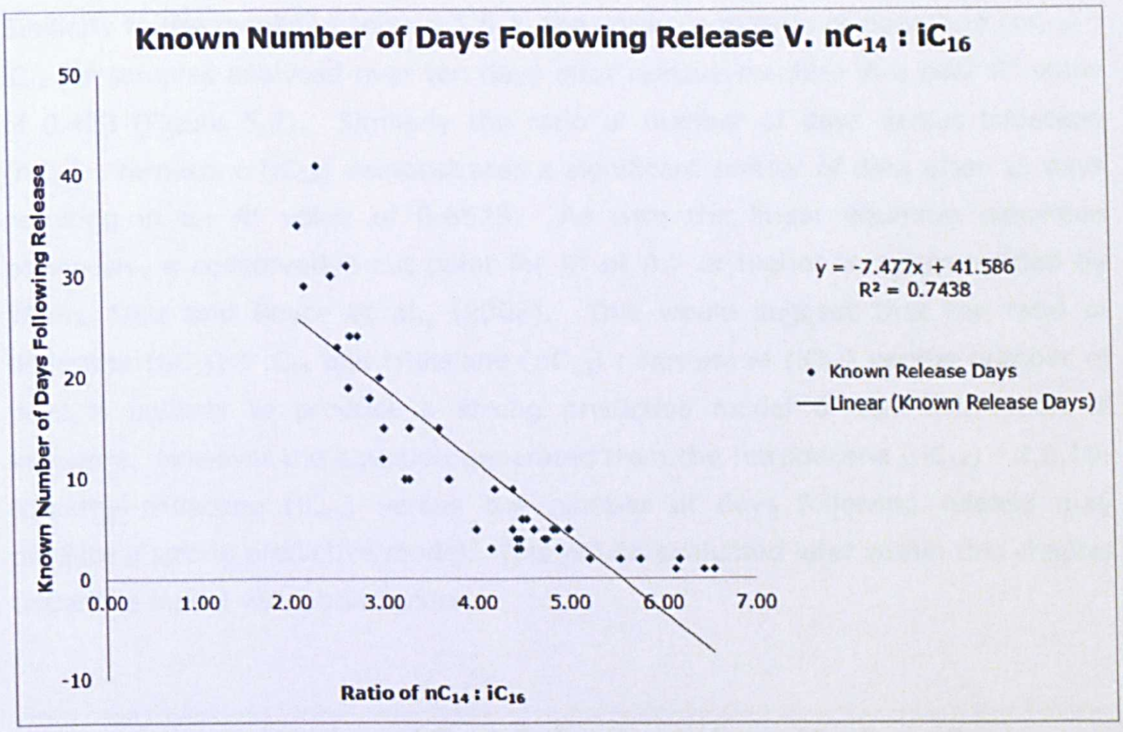


Figure 5.2 Linear Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Dodecane (nC_{12}) to iC_{14}, Tridecane (nC_{13}) to Farnesane (iC_{15}) and Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

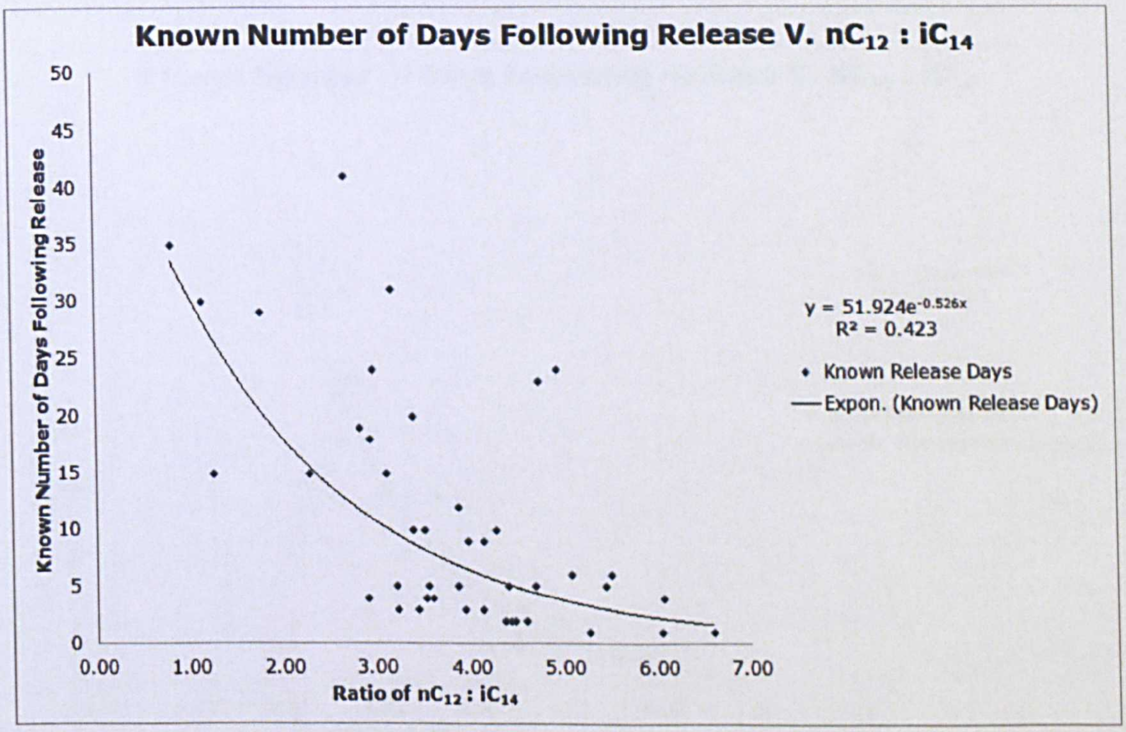
5.5.2 Exponential Regression Model

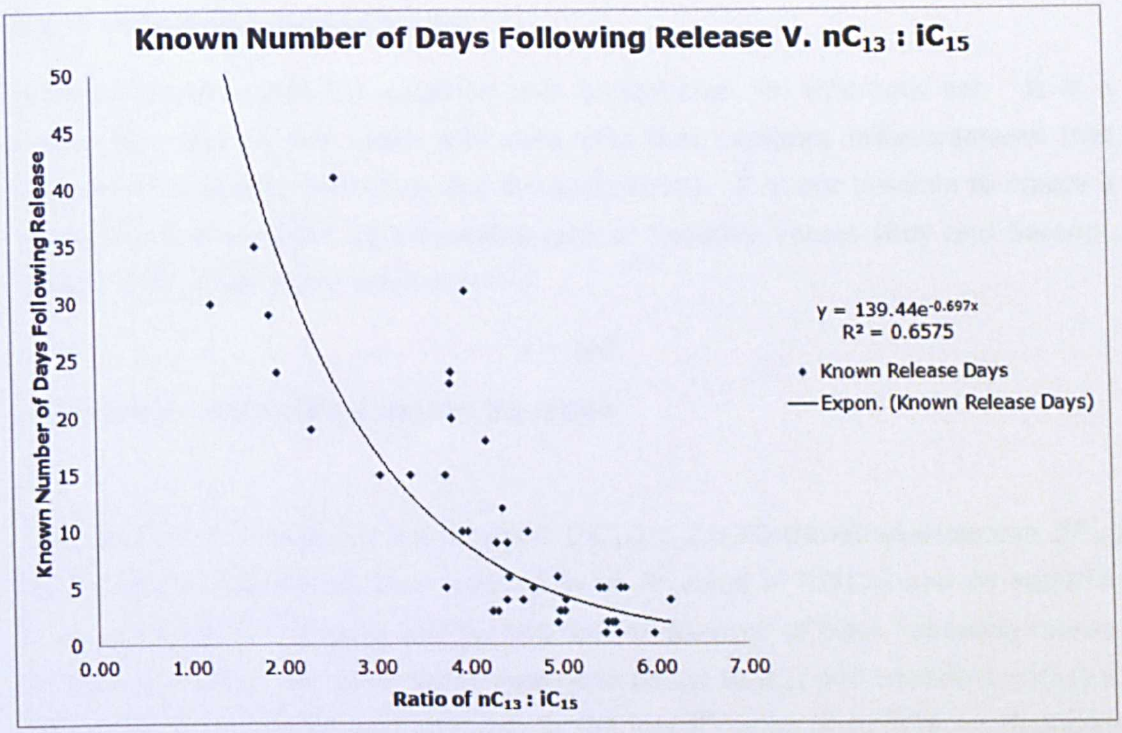
The resulting R^2 value and the exponential equation calculated from the ratio of dodecane (nC_{12}) : iC_{14}, tridecane (nC_{13}) : farnesane (iC_{15}) and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release are presented in Figure 5.3. Similar to the evaporation linear regression distribution described previously the highest value for R^2 was determined for tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release which was found to generate an R^2 value of 0.9187 with an equation of:

$$y = 192.29e^{-0.814x}$$

Equation 8 Exponential Regression Equation

Similarly to the results in section 5.5.1, the variance in ratio of dodecane (nC_{12}) : iC_{14} for samples analysed over ten days after release resulted in a poor R^2 value of 0.423 (Figure 5.3). Similarly the ratio of number of days versus tridecane (nC_{13}) : farnesane (iC_{15}) demonstrates a significant scatter of data after 15 days resulting in an R^2 value of 0.6575. As with the linear equation described previously, a conservative cut point for R^2 of 0.7 or higher is recommended by Wiens, Dale and Boyce et al., (2008). This would suggest that the ratio of dodecane (nC_{12}) : iC_{14} and tridecane (nC_{13}) : farnesane (iC_{15}) versus number of days is unlikely to produce a strong predictive model of age estimation of kerosene. However the equation generated from the tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release may produce a strong predictive model. This will be evaluated later within this chapter under the model validation section.





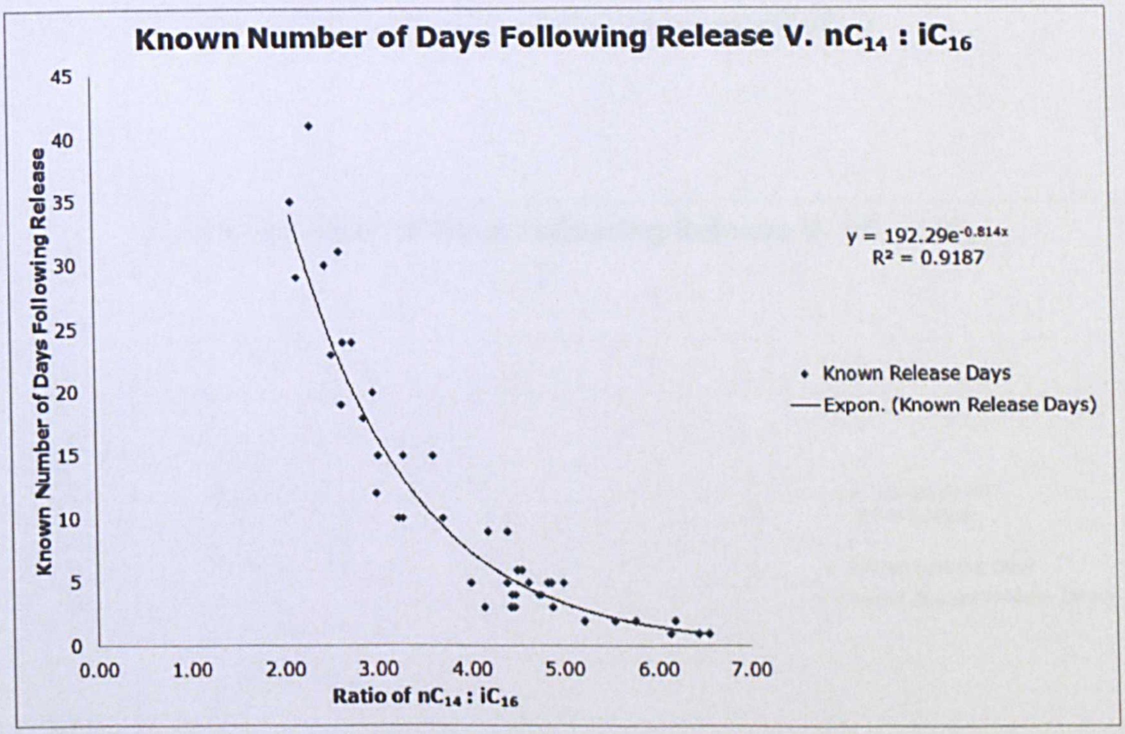


Figure 5.3 Exponential Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Dodecane (nC_{12}) to iC_{14}, Tridecane (nC_{13}) to Farnesane (iC_{15}) and Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

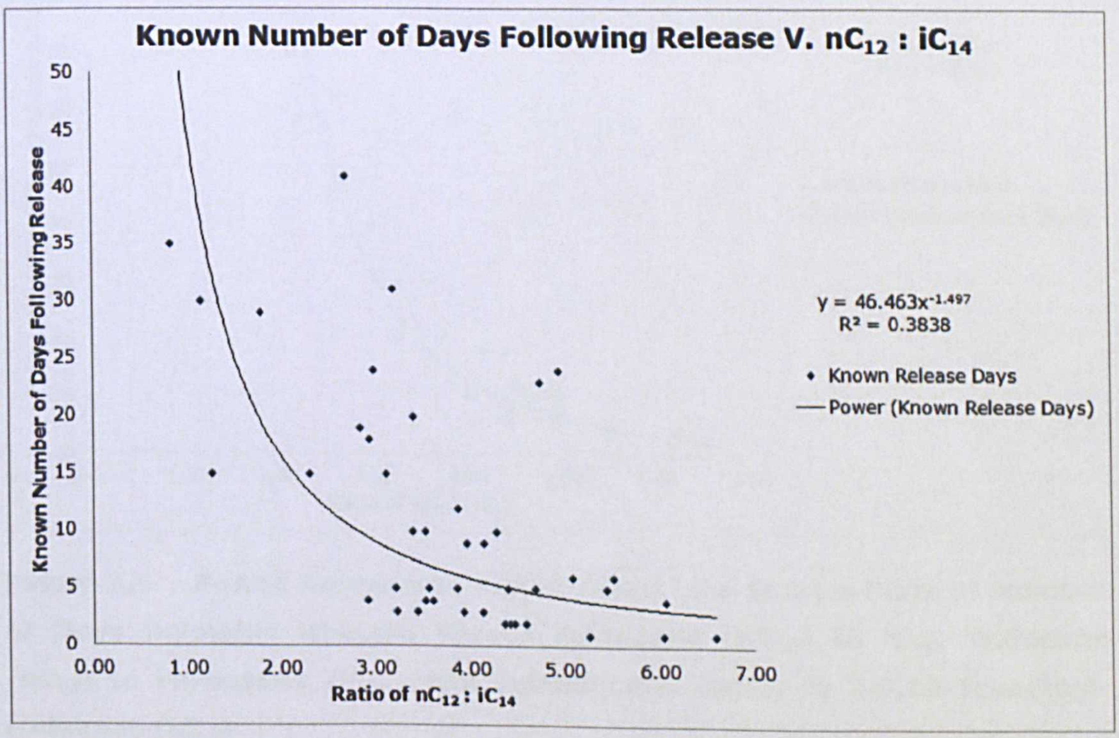
5.5.3 Power Regression Model

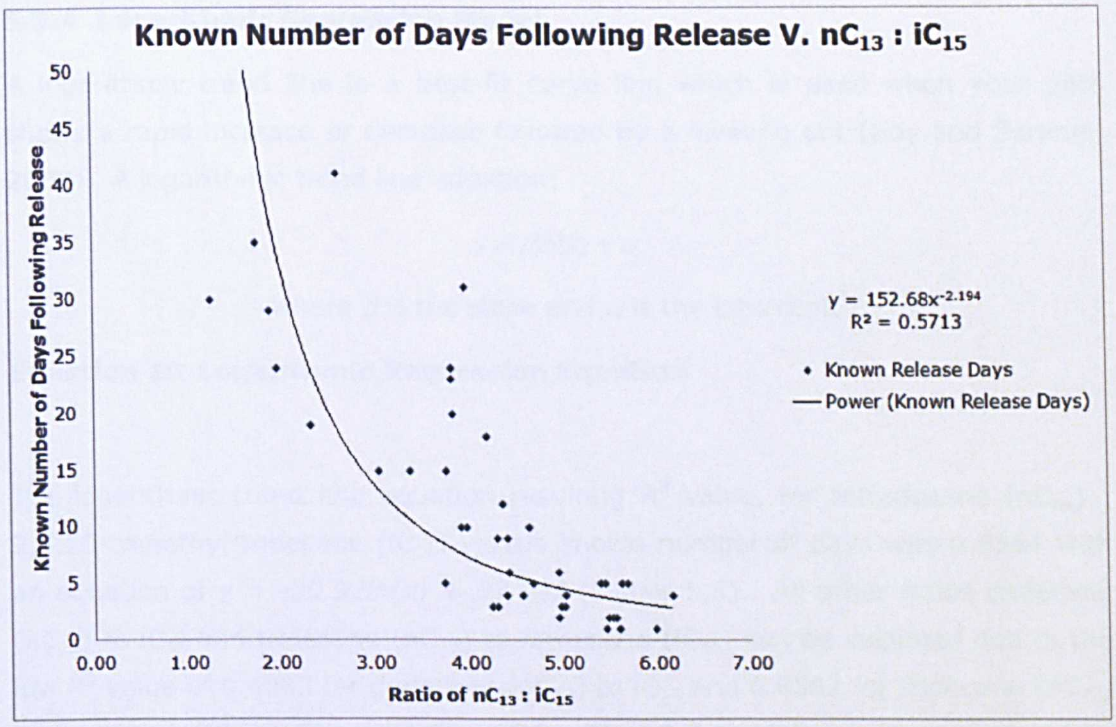
A power trend regression equation was constructed for this data set. It is a curved line that is best used with data sets that compare measurements that increase at a specific rate (Roy and Sarangi, 2008). It is not possible to create a power trend line if your data contains zero or negative values (Roy and Sarangi, 2008). The power trend line equation:

$$y = \alpha x^{\beta}$$

Equation 9 Power Regression Equation

The resulting R^2 value, for tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus known number of days resulted in an R^2 value of 0.9116 and an equation of $y = 514.43x^{-3.165}$ (Figure 5.4) for the ratio of Number of Days Following release versus nC_{14} : iC_{16}. All other ratios dodecane (nC_{12}) to iC_{14} and tridecane (nC_{13}) to farnesane (iC_{15}) can be excluded due to the low R^2 value of 0.3838 for dodecane (nC_{12}) to iC_{14} and 0.5713 for tridecane (nC_{13}) to farnesane (iC_{15}).





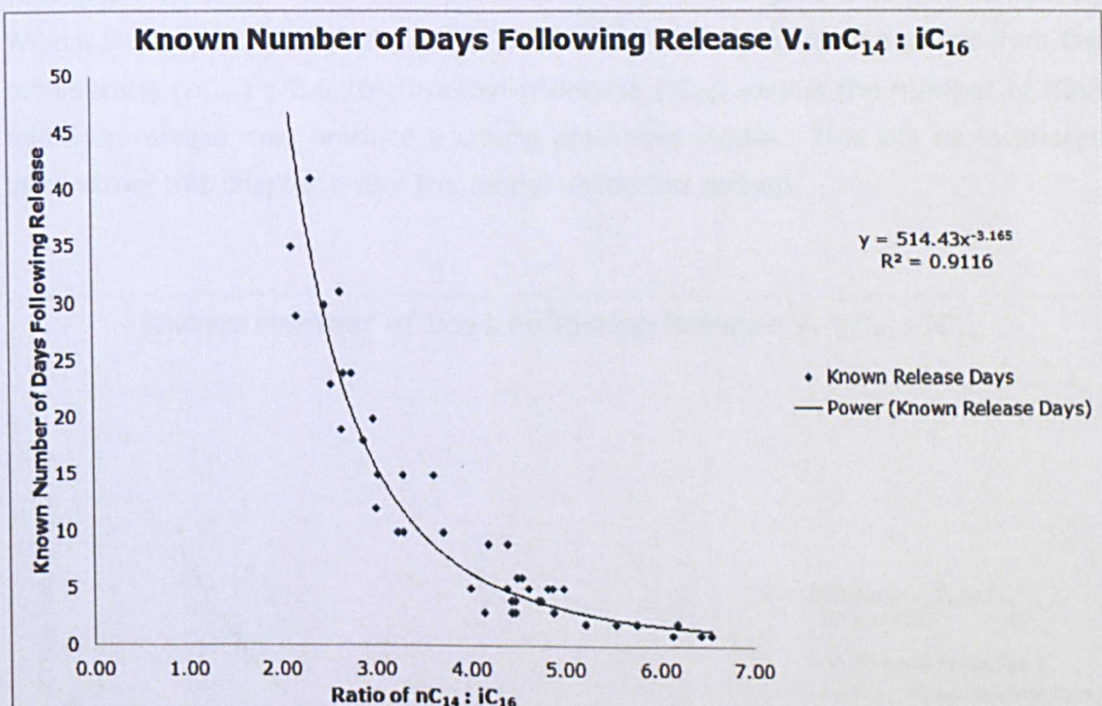


Figure 5.4 Power Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Dodecane (nC_{12}) to iC_{14}, Tridecane (nC_{13}) to Farnesane (iC_{15}) and Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

5.5.4 Logarithmic Regression Model

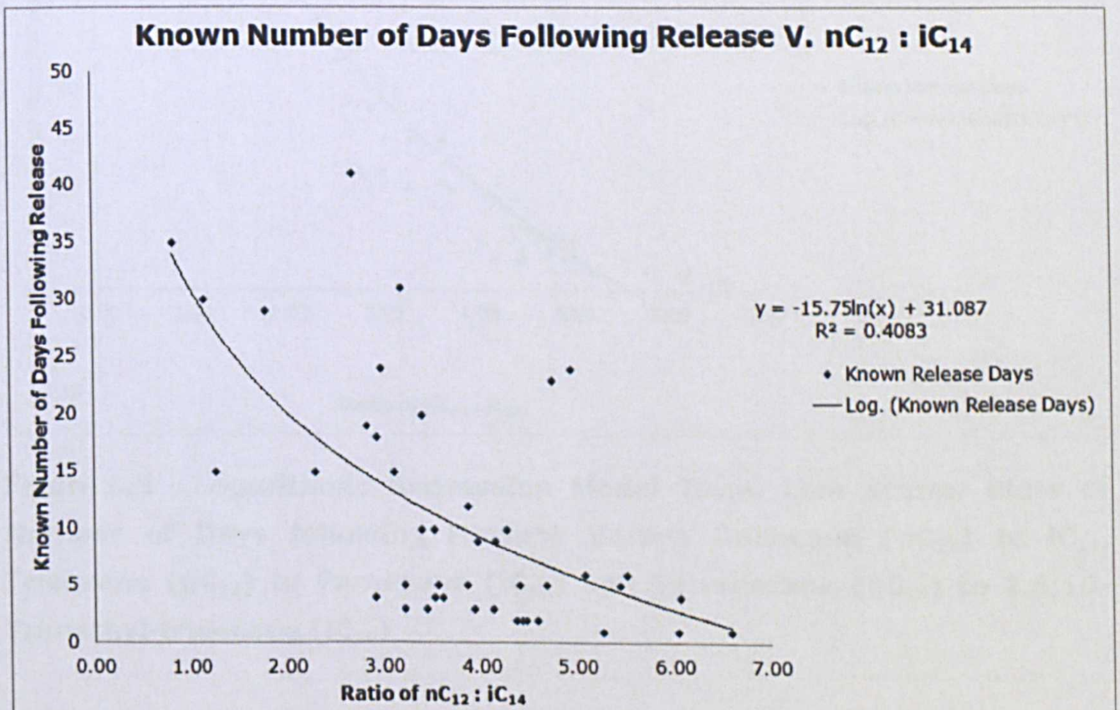
A logarithmic trend line is a best-fit curve line which is used when your data shows a rapid increase or decrease followed by a leveling out (Roy and Sarangi, 2008). A logarithmic trend line equation:

$$y = \beta \ln(x) + \alpha$$

where β is the slope and α is the intercept.

Equation 10 Logarithmic Regression Equation

The logarithmic trend line equation resulting R^2 value, for tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus known number of days was 0.8354 with an equation of $y = -30.92ln(x) + 53.142$ (Figure 5.5). All other ratios dodecane (nC_{12}) to iC_{14} and tridecane (nC_{13}) to farnesane (iC_{15}) can be excluded due to the low R^2 value of 0.4083 for dodecane (nC_{12}) to iC_{14} and 0.6562 for tridecane (nC_{13}) to farnesane (iC_{15}). The cut off point for R^2 of 0.7 or higher is recommended by Wiens, Dale and Boyce et al., (2008). However the equation generated from the tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release may produce a strong predictive model. This will be evaluated later within this chapter under the model validation section.



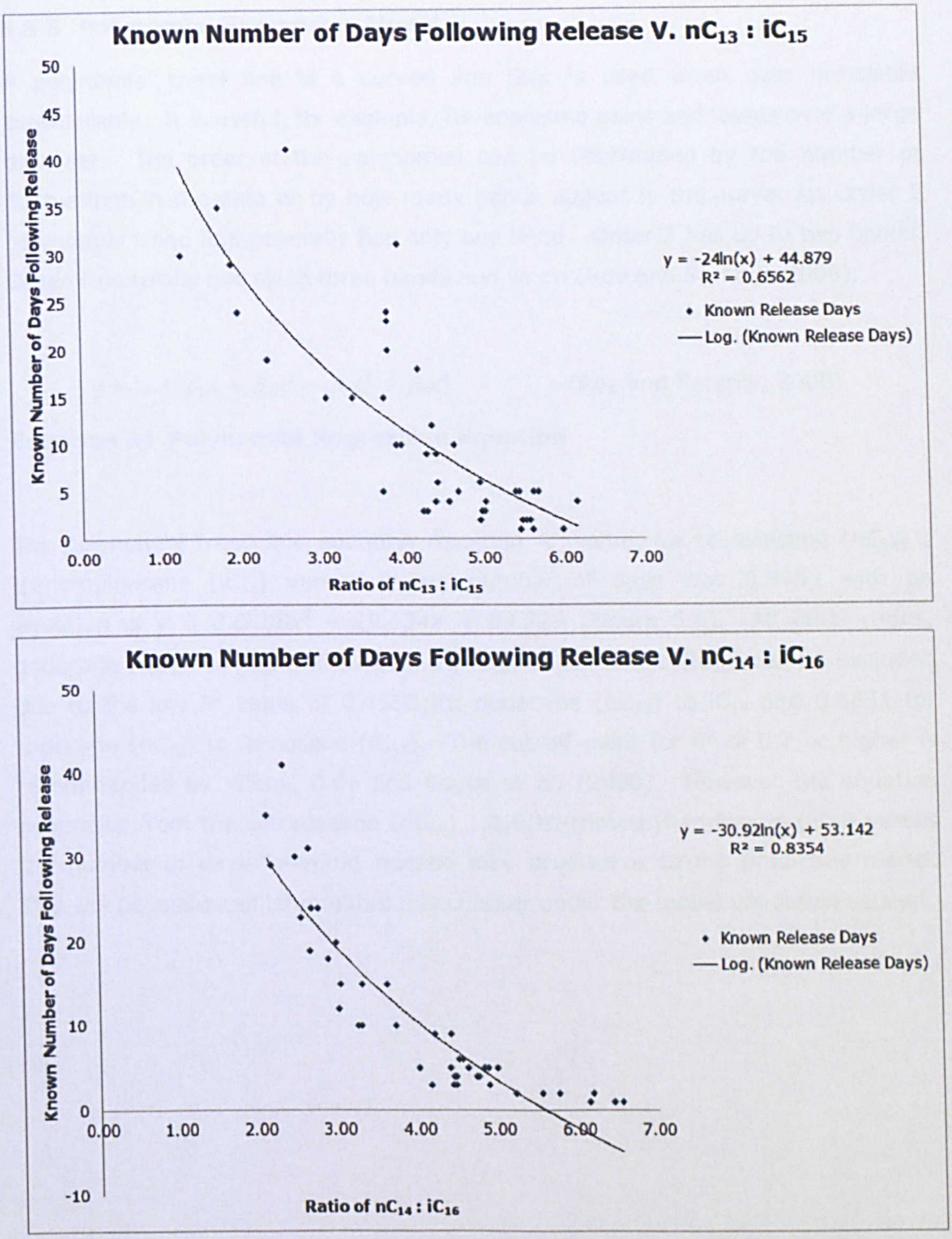


Figure 5.5 Logarithmic Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Dodecane (nC_{12}) to iC_{14}, Tridecane (nC_{13}) to Farnesane (iC_{15}) and Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

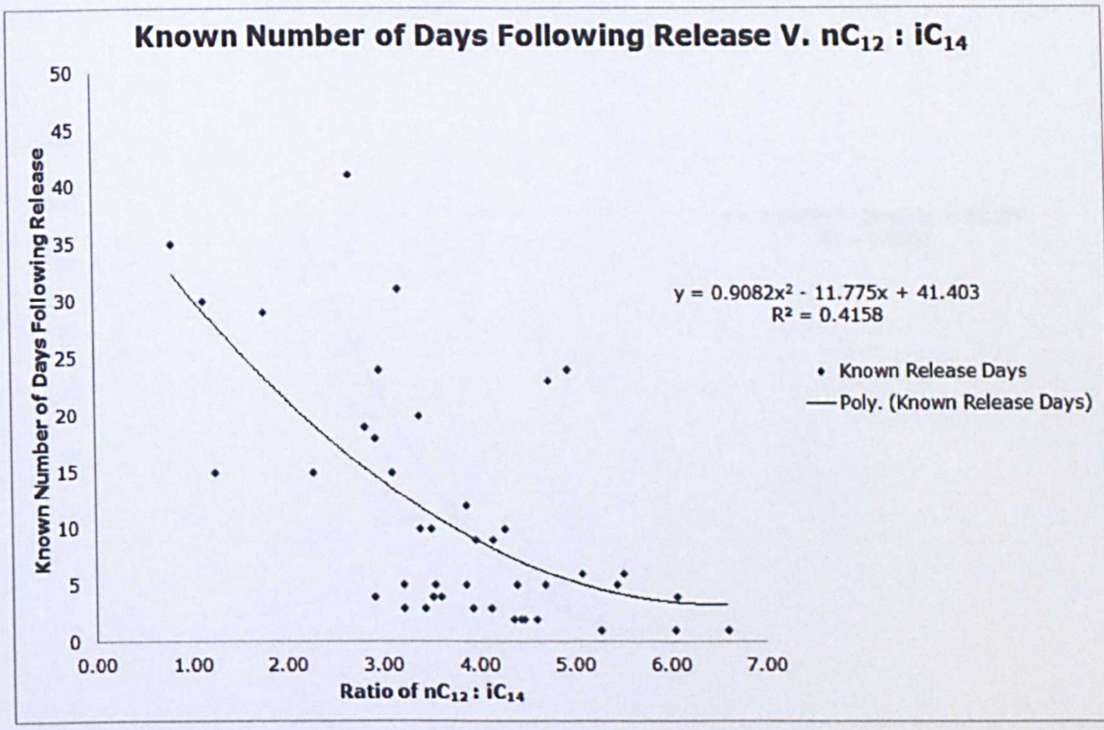
5.5.5 Polynomial Regression Model

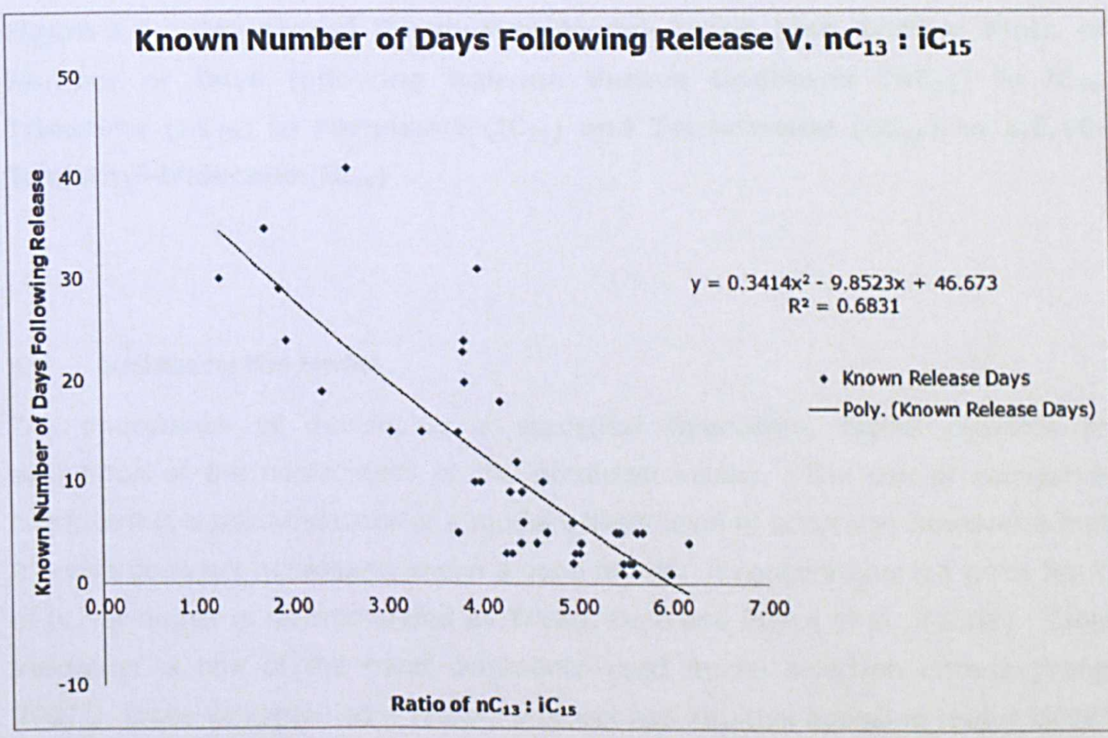
A polynomial trend line is a curved line that is used when data fluctuates considerably. It is useful, for example, for analysing gains and losses over a large data set. The order of the polynomial can be determined by the number of fluctuations in the data or by how many bends appear in the curve. An Order 2 polynomial trend line generally has only one bend. Order 3 has up to two bends. Order 4 generally has up to three bends and so on (Roy and Sarangi, 2008).

$$y = \alpha + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \beta_4 x^4 \qquad \text{(Roy and Sarangi, 2008)}$$

Equation 11 Polynomial Regression Equation

The polynomial trend line equation resulting R^2 value, for tetradecane (nC_{14}) : trimethyldecane (iC_{16}) versus known number of days was 0.8952 with an equation of $y = 2.6408x^2 - 29.634x + 84.229$ (Figure 5.6). All other ratios, dodecane (nC_{12}) to iC_{14} and tridecane (nC_{13}) to farnesane (iC_{15}) can be excluded due to the low R^2 value of 0.4158 for dodecane (nC_{12}) to iC_{14} and 0.6831 for tridecane (nC_{13}) to farnesane (iC_{15}). The cut-off point for R^2 of 0.7 or higher is recommended by Wiens, Dale and Boyce et al. (2008). However the equation generated from the tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release may produce a strong predictive model. This will be evaluated later within this chapter under the model validation section.


Known Number of Days Following Release V. nC12 : iC14
y = 0.9082x² - 11.775x + 41.403
R² = 0.4158
Known Release Days
Poly. (Known Release Days)
Known Number of Days Following Release
Ratio of nC12 : iC14
0
5
10
15
20
25
30
35
40
45
50
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00


Known Number of Days Following Release V. nC13 : iC15
y = 0.3414x² - 9.8523x + 46.673
R² = 0.6831
Known Release Days
Poly. (Known Release Days)
Known Number of Days Following Release
Ratio of nC13 : iC15
-10
0
10
20
30
40
50
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00

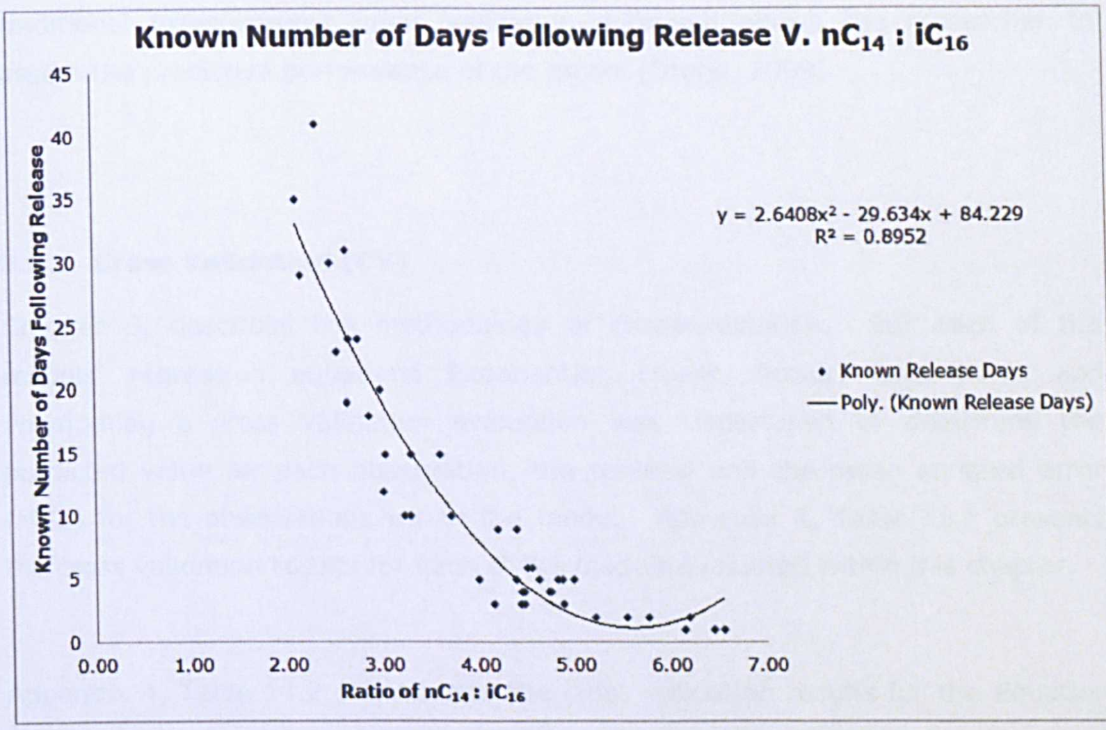


Figure 5.6 Polynomial Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Dodecane (nC_{12}) to iC_{14}, Tridecane (nC_{13}) to Farnesane (iC_{15}) and Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

5.6 Validating the Model

The importance of developing a statistical forecasting model requires an evaluation of the correctness of the predicted values. The use of correlation coefficient is a good indicator of a model's likely level of accuracy; however a high R^2 value does not necessarily mean a good model. A conservative cut point for R^2 of 0.7 or higher is recommended by Wiens, Dale and Boyce et al., (2008). Cross validation is one of the most commonly used model selection criteria (Yang, 2007). Cross validation as a testing protocol has intuitive appeal in that it closely mimics the actual forecast situation using data which are similar to those which would be encountered in forecasting (Michaelsen, 1987). Cross validation is a statistical re-sampling technique used to test the strength of a model prediction (Chung and Fabbri, 2008). In typical cross-validation, the training and validation sets must cross-over in successive rounds such that each data point has a chance of being validated against (Refaeilzadeh, Tang and Liu, 2008). The idea of this traditional leave-one-out cross validation approach allows the researcher to assess the predictive performance of the model (Droge, 1999).

5.6.1 Cross Validation (CV)

Chapter 3, describes the methodology of cross-validation. For each of the models' regression equations Exponential, Linear, Power, Logarithmic and Polynomial, a cross validation evaluation was undertaken to determine the predicted value for each observation, the residual and the mean squared error (MSE) for the observations within the model. Appendix 4, Table 11.1 presents the cross validation results for each of the models evaluated within this chapter.

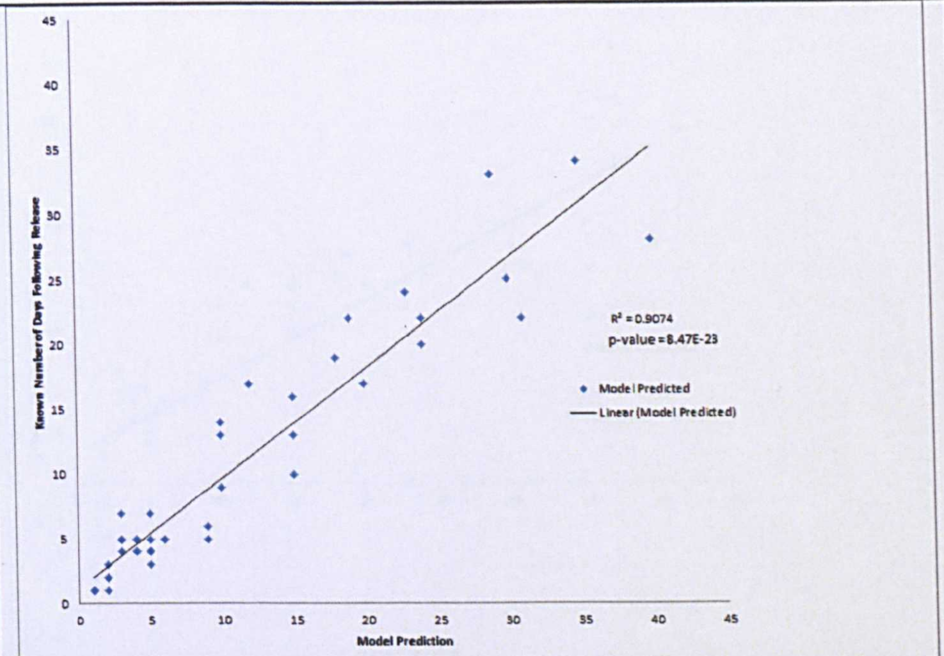
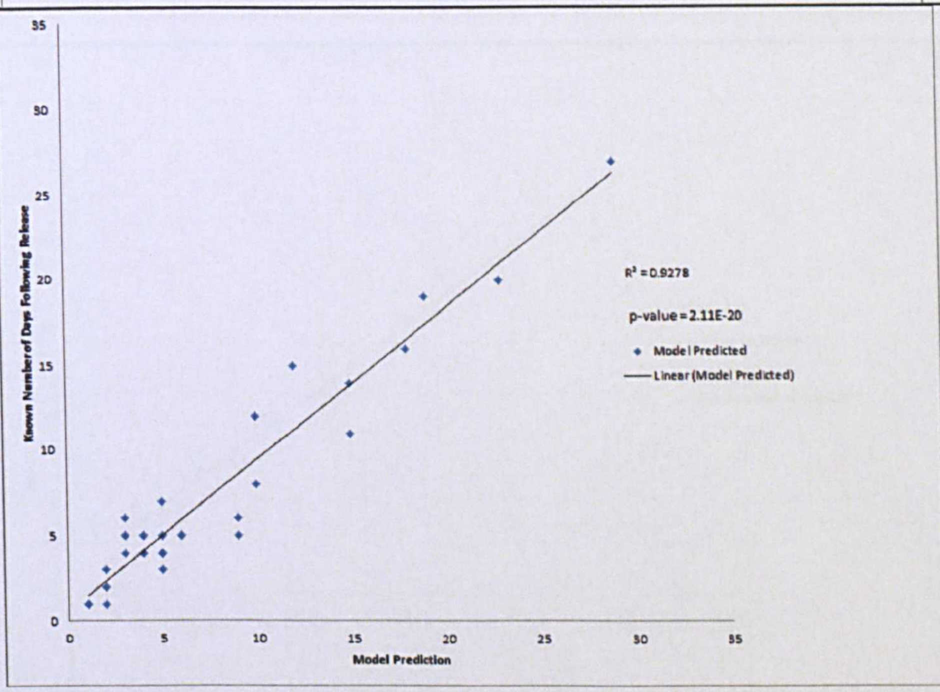
Appendix 4, Table 11.2 summarizes the cross validation results for the Equation based on Christensen and Larsen 1993 study using the equation generated by Kaplan et al., (1996b). Similarly the Hurst and Schmidt (2005) equation was assessed using the cross-validation technique and the findings are presented in Appendix 4, Table 11.3. Appendix 4, Table 11.4 to Table 11.7 presents the cross-validation results when sample numbers were eliminated. In section 3.15.3 of Chapter 3 the rationale behind the elimination of sample numbers is explained. Finally Table 5.6 includes a summary of the ANOVA, %RSD, R^2 for the Plot of Known Number of Days Following Release versus Predicted Number of Days Following Release for each model and the Christensen and Larsen Linear Regression and Hurst Linear Regression Models, all of the mean squared error (MSE) and adjusted mean squared error results for each of the models is summarised.

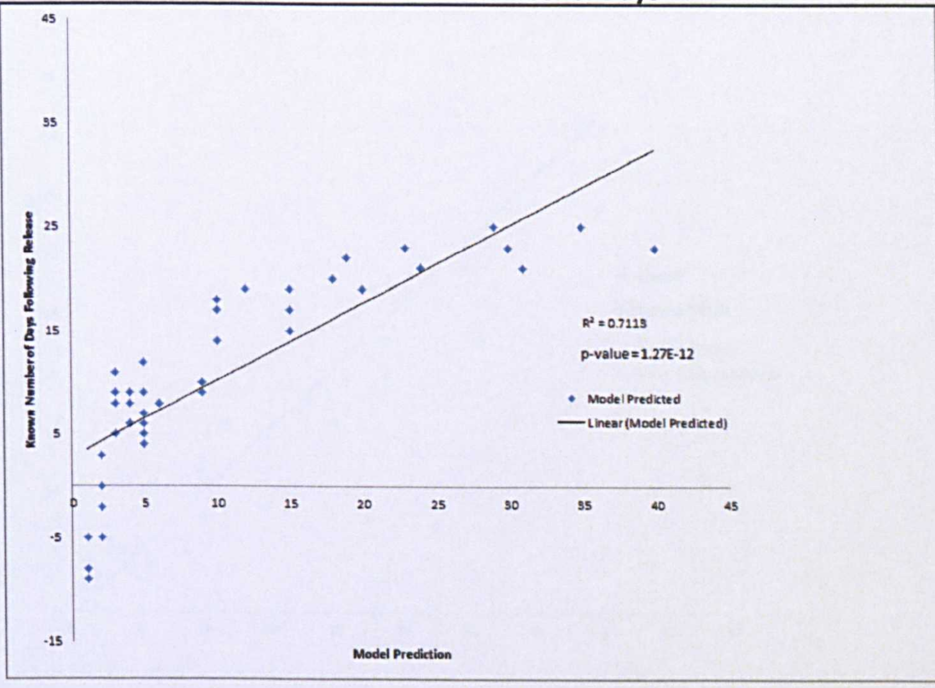
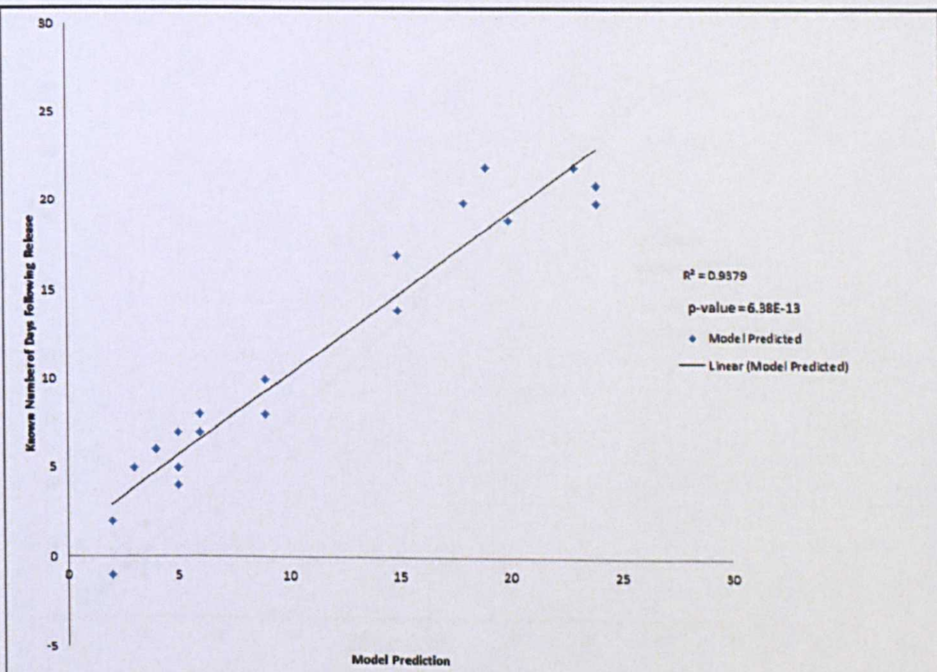
Appendix 4, Table 11.4 to Table 11.7 present the results of a reassessment of the cross validations, applying the omission exercise described in section 3.15.2 of the methodology chapter. The final model prediction estimate in each of the tables is determined by the uses of the equation which includes all of the data used to generate the model.

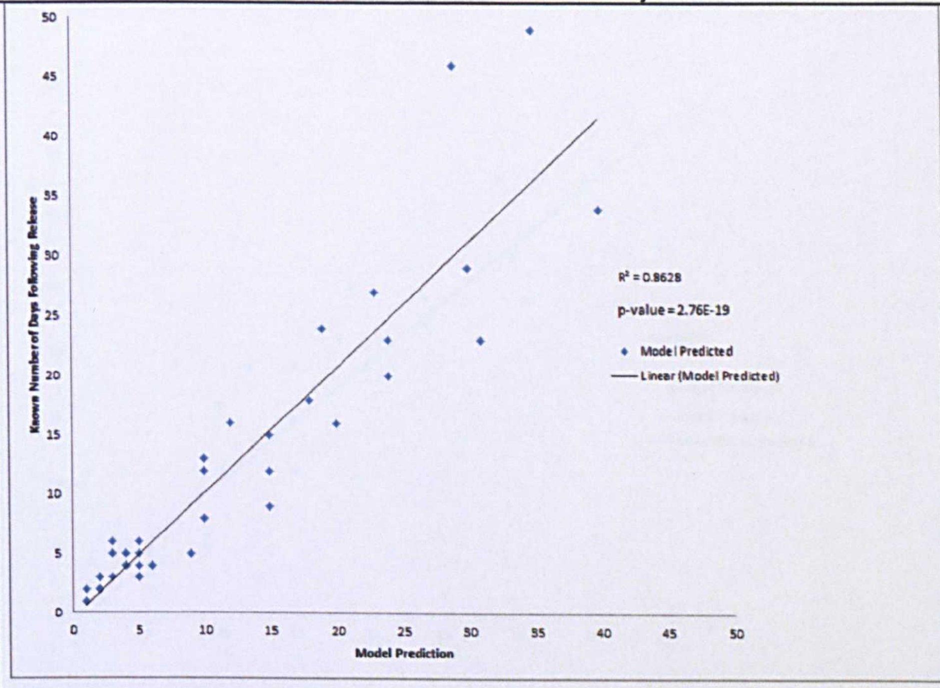
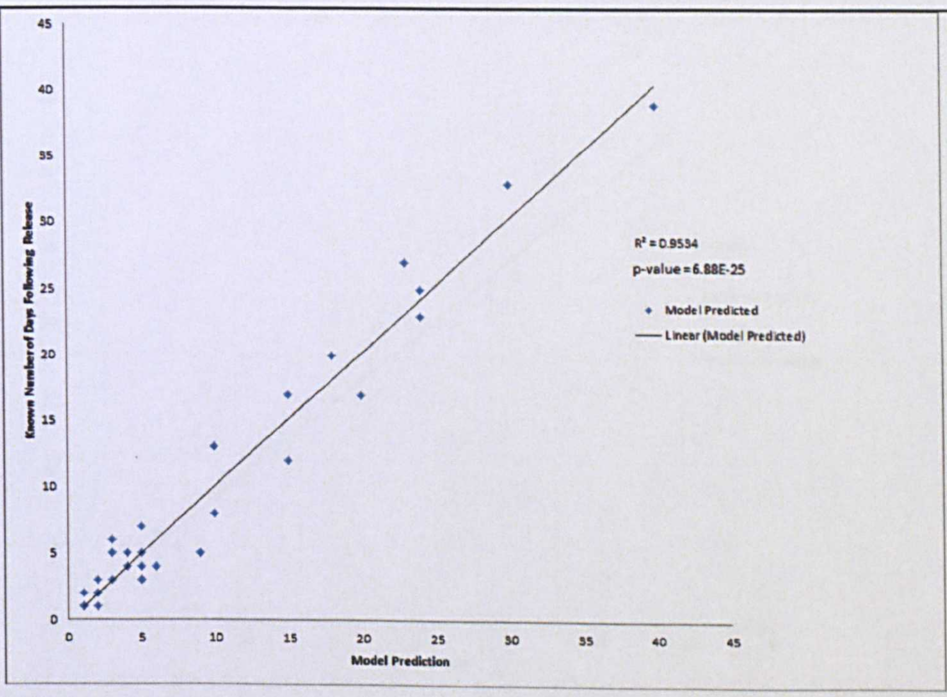
Table 5.6 presents the calculated R^2 values which have been determined from a linear regression plot of the known number of days following release against the final models predicted estimated number of days (Figure 5.7) for each of the models used. The adjusted R^2 represents the omission of a number of samples from the dataset. The statistics from the analysis of variance (ANOVA) test for each model was undertaken and the results were presented in Table 5.6. The p-value is the probability that the variation between conditions may have occurred by chance whereas the *f* value is the variance within the group mean. The percentage relative standard deviation (%RSD) is a measure of the precision of the predicted measures before and after the omission of samples from the data set.

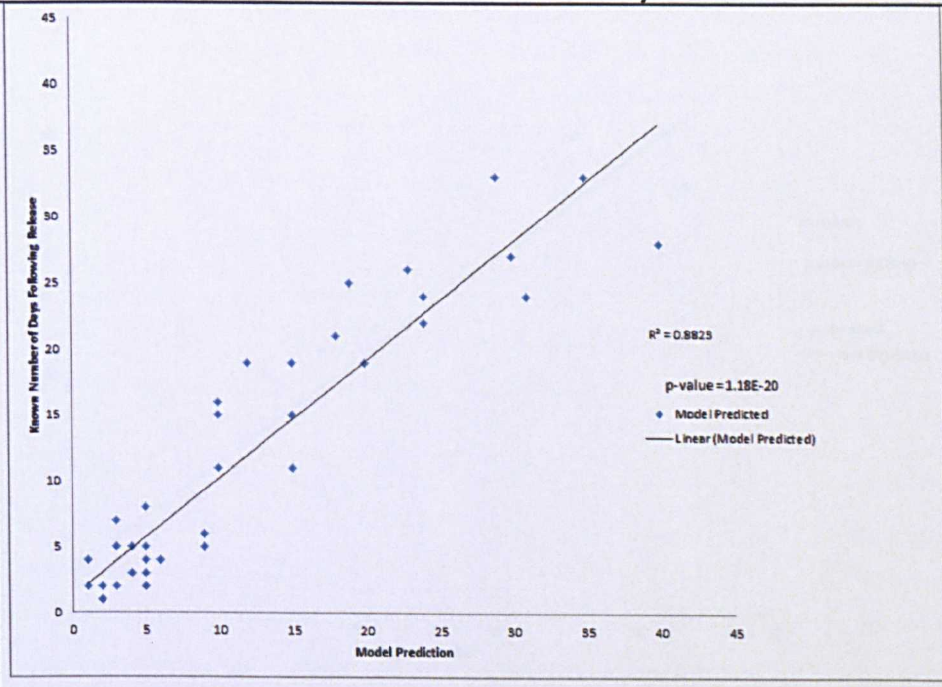
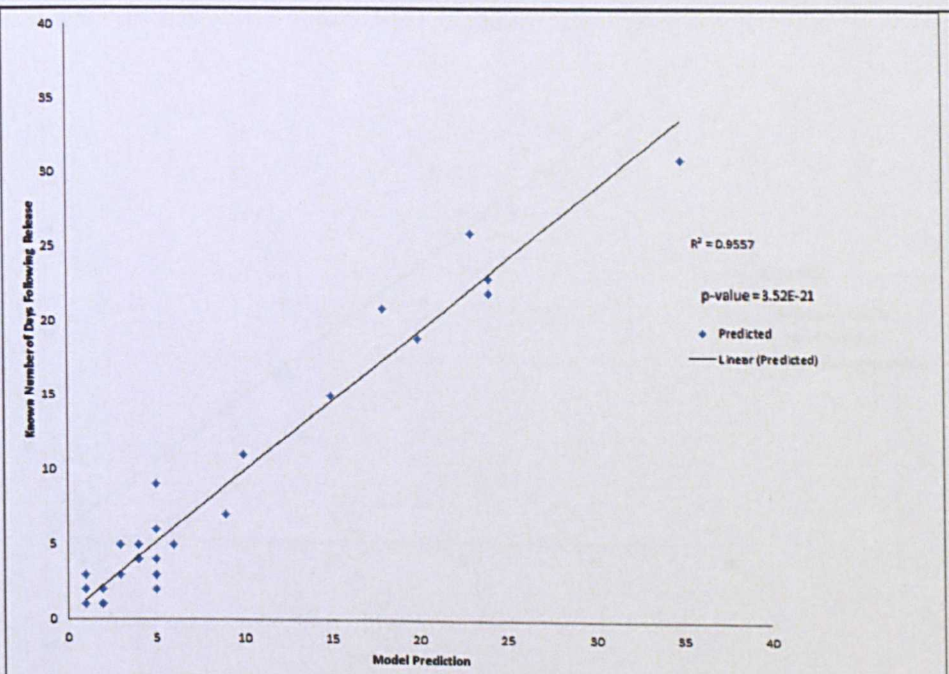
Table 5.6 Summary of MSE, ANOVA, %RSD and R^2 for the Plot of Known Number of Days Following Release versus Predicted Number of Days Following Release for Each Model and the Christensen and Larsen Linear Regression and Hurst Linear Regression Models

Model	R^2	MSE	Count	ANOVA *P-Value*	%RSD
Exponential	0.9074	12	43	8.47E-23	87
Reduced Exponential	0.9278	3	35	2.11E-20	86
Linear	0.7113	32	43	1.27E-12	83
Reduced Linear	0.9379	3	19	6.38E-13	68
Power	0.8628	18	43	2.76E-19	94
Reduced Power	0.9534	3	36	6.88E-25	91
Polynomial	0.8823	13	43	1.18E-20	88
Reduced Polynomial	0.9557	3	31	3.52E-21	103
Christensen and Larsen Linear Regression Model	0.8325	5	12	3.49E-05	
Hurst Linear Regression Model	0.9401	3	33	1.63E-20	

Model	Known Number of Days Following Release Versus Model Predicted Number of Days
Exponential	
Reduced Exponential	

<table>
<tr><th>Model</th><th>Known Number of Days Following Release
Versus
Model Predicted Number of Days</th></tr>
<tr><td>Linear</td><td>
</td></tr>
<tr><td>Reduced Linear</td><td>
</td></tr>
</table>

<table>
<tr><th>Model</th><th>Known Number of Days Following Release
Versus
Model Predicted Number of Days</th></tr>
<tr><td>Power</td><td>
</td></tr>
<tr><td>Reduced Power</td><td>
</td></tr>
</table>

<table>
<tr><th>Model</th><th>Known Number of Days Following Release
Versus
Model Predicted Number of Days</th></tr>
<tr><td>Polynomial</td><td>
</td></tr>
<tr><td>Reduced Polynomial</td><td>
</td></tr>
</table>

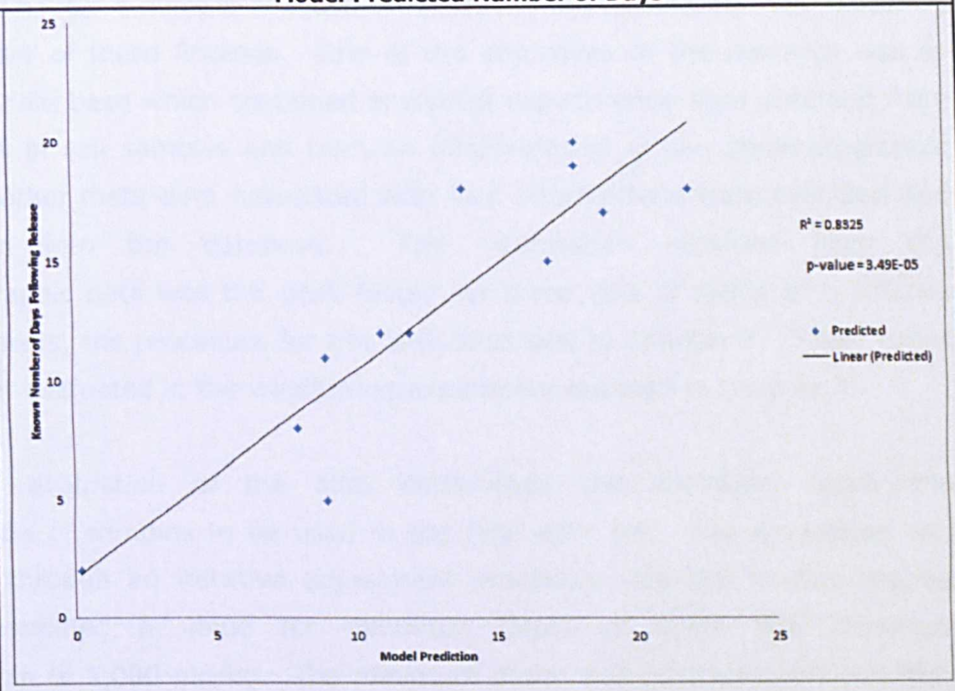
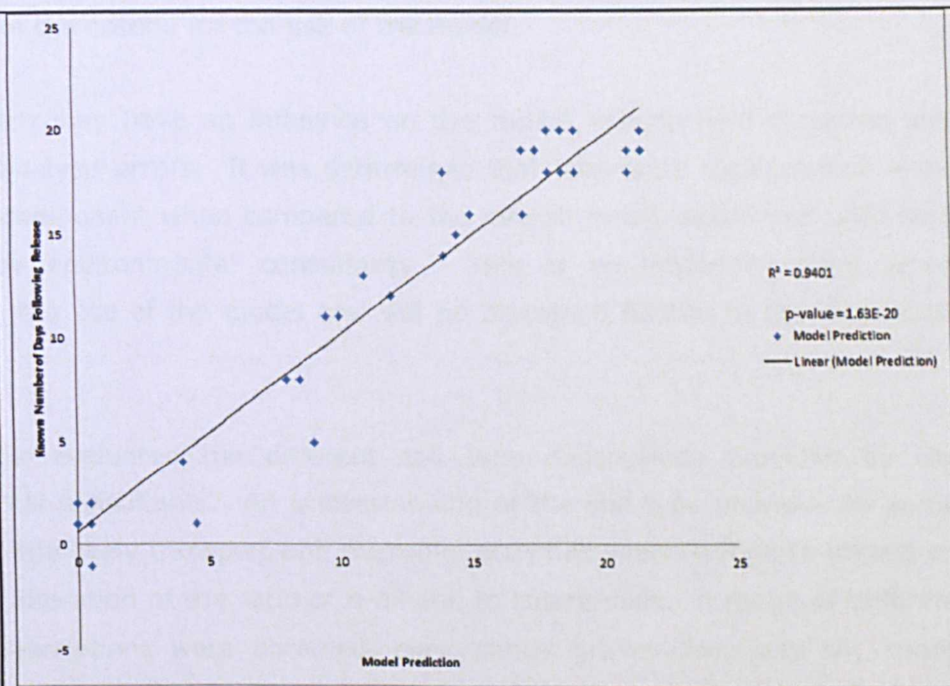
<table>
<tr><th>Model</th><th>Known Number of Days Following Release
Versus
Model Predicted Number of Days</th></tr>
<tr><td>Christensen and Larsen Linear Regression Model</td><td>
</td></tr>
<tr><td>Hurst Linear Regression Model</td><td>
</td></tr>
</table>

Figure 5.7 Known Number of Days Versus Predicted Number of Days for Each Model

5.6.2 Chapter 5 Summary of Findings

Within this Chapter a number of research questions were explored. The following is a summary of these findings. One of the objectives of the research was to establish a data base which contained analytical experimental data obtained from the analysis of soil samples and from an interpretation of the chromatographic data. The other meta-data associated with field observations were collected and documented into the database. The information obtained from the chromatographic data was the peak height for three sets of ratios of n-alkanes and isoprenoids, the procedure for this was described in Chapter 3. These ratios were further evaluated in the weathering experiment outlined in Chapter 4.

The initial evaluation of the data determined the minimum depth and concentration of samples to be used in the final data set. This evaluation was conducted through an iterative adjustment procedure describe in this chapter which determined a value for minimum depth of 0.4m and minimum concentration of 1,000 mg/kg. The minimum depth and minimum concentration will be one of the criteria for the use of the model.

Factors which may have an influence on the model include field sampling and laboratory analysis errors. It was determined that laboratory experimental error is a minor component when compared to the overall errors associated with field sampling by environmental consultants. This is an important point when considering the use of the model and will be discussed further in the discussion chapter.

This chapter evaluated the different soil type descriptions provided by the environmental consultants. An understanding of the soil type provides for some insight into the likely transport and microbial activities which will go to impact on the rate of alteration of the ratio of n-alkane to isoprenoids. A range of different soil type descriptions were obtained, clay, sandy gravel clay, clay silt, made ground fill, topsoil and clay with peat. The soil moisture content varied for each soil type described. The type of soil and percentage moisture content was found to have an impact on the rate of alteration of the diagnostic ratios used.

Regression model equations were used to describe the relationship between the diagnostic ratio versus number of days from release. With the use of coefficient of determination R-squared value (R^2) each model was assessed. For each of the five different models evaluated it was found that the known number of days following release versus the ratio of nC_{14} : iC_{16} was found to describe the data the best. The plots of the other ratios (nC_{12} : iC_{14} and nC_{13} : iC_{15}) returned poor R-squared value values. It would appear that these ratios may be more susceptible to the mechanism of evaporation.

For each of the models' regression equations Exponential, Linear, Power, Logarithmic and Polynomial, a cross validation evaluation was undertaken to determine the predicted value for each observation, the residual and the mean squared error (MSE) for the observations within the model. The power regression model provided the best correlation between the model prediction and the known number of days following release of an R^2 value of 0.9625 (adjusted). The model cross validation evaluation was extended to the Hurst, Christensen and Larsen models for comparison.

6.0 Chapter 6 Discussion

In the preceding two chapters the research findings have been presented. This chapter has three primary functions. Firstly, this chapter of the dissertation acts as an aide to the reader, providing an overview of the research undertaken, including a statement of the research questions. Following this, is a summary and discussion of the six study aims and objectives set out in Chapter 1. Finally, a discussion of the findings to elicit criteria constraining the conditions within which the kerosene spill ageing equation can be relied upon to estimate kerosene releases will be presented and discussed within this chapter.

Presented in Chapter 2, the literature review provides a background to the existing theoretical concepts related to oil spill mechanisms and the chemical composition of kerosene. It is important to relate the research findings within this study to existing theoretical concepts. The literature review, therefore, constitutes an additional data source to support the overall research findings. The chapter identifies the theories which are relevant to this research place in context the validity and overall quality of the findings of this research.

6.1 Research Questions

This research has been driven by the industry-led research questions outlined in Chapter 1. It was anticipated that this research would develop and validate a model to predict the age of kerosene spills in typical UK and Irish environmental conditions. The model would be based on normal alkane / isoprenoid ratios extracted from several chromatograms. The industrial-led research questions outlined in Table 6.1 were raised in Chapter 1. The research aims and objectives have been successfully addressed in the findings which are presented in Chapters 4 and 5. These research questions will be discussed further within this chapter in an effort to contribute to an enhanced understanding of the appropriate criteria associated with the use of the selected model, which will be used to age the release of kerosene oil in home heating oil spills. It is important to note the limitations of the use of the final model in an effort to allow the end user to fully understand the constraints of its use. Within this chapter a critical evaluation of its use will be discussed.

Table 6.1 Research Aim and Objectives

Number	Research Questions
1	Evaluate the preferential depletion of the n-alkanes dodecane (nC_{12}), tridecane (nC_{13}), tetradecane (nC_{14}) and pentadecane (nC_{15}) to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil by conducting a series of artificial weathering experiments.
2	Develop an analytical method for the rapid extraction of isoprenoid and n-alkane content from chromatographic data.
3	Develop a database of chromatograms and associated meta data obtained from the analysis of kerosene spill samples (soil and water) received for analysis between 2009 and 2011 at Jones Environmental Forensics Limited laboratory. These samples have been submitted for analysis by environmental consultants working on domestic home heating oil releases in Ireland and the United Kingdom.
4	Analyse the database of isoprenoid and n-alkane data of kerosene spill samples (soil). Evaluate the environmental factors (weathering and biodegradation) affecting isoprenoids and n-alkane ratios in post-spill kerosene in soil in order to accurately date a spill event.
5	Develop an equation equivalent to the Christensen and Larsen (1993), Kaplan, Galperin, Alimi et al., (1996b) equation used for middle distillates but based on forensic markers of isoprenoids and n-alkanes present in kerosene, which is applicable to kerosene spills.
6	Elicit criteria which constrain the conditions within which the kerosene spill dating equation can be relied upon.

6.2 Research Question – Analysis of Kerosene and Weathering Experiment

The first research question sought to evaluate the preferential depletion of the n-alkanes: dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$), when compared to the isoprenoids: iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in kerosene oil, by conducting a series of artificial weathering experiments (Chapter 4). The main limitation of the weathering experiment was that the evaporation study was conducted on neat oil and not on soil impacted by oil. Future research on laboratory trials using various soil types would enhance the use of the model. The rate of alteration of the ratio can be influenced by the matrix type among other parameters which was described in Chapter 2 (Atlas and Bartha, 1992; Kaplan et al., 1997). In section 3.4 and 3.5 of Chapter 3 the use of gas chromatography utilising both flame ionisation detection and mass spectrometry techniques was discussed. It was stated that effective separation was achieved using a suitable analytical chromatography column where the compounds present in kerosene would elute in order of volatility with the most volatile eluting first (Jones, 2003). Furthermore, separation was achieved by a combination of factors such as boiling point and molecular weight of compounds. Having listed the analytical instrumental factors required to obtain a chromatographic profile of kerosene, a standard of a mixture of n-alkanes from octane (nC_8) to tetracontane (nC_{40}) and a polycyclic aromatic hydrocarbon (PAH) standard mixture were analysed to characterise the retention time characteristics of the methodologies employed. This provided a high degree of confidence that the unique profile resulting from the analysis of kerosene oil was achieved. Reflecting on these findings, it was useful to draw upon the unique power of mass spectrometry in its ability to confirm the presence of the recalcitrant isoprenoid branched aliphatic and n-alkane compounds found in the analysis of a known source of kerosene oil. This provided a high degree of confidence when selecting the required n-alkanes and isoprenoids in the data collection stage of the research. Other researcher (Wang and Stout, 2007) have described the use of GC-FID and GC-MS as powerful tool for the fingerprinting of oils.

Furthermore, presented in Chapter 4 was the use of selective ion monitoring which is a mass spectrometry tool used to elucidate compound structures. The mechanism by which mass spectrometry operates to achieve these ion fragmentograms was described in detail in section 3.6 of Chapter 3. The findings indicate this to be a particularly powerful tool as it was possible to accurately identify each of the n-alkanes and isoprenoids found in kerosene by targeting ions with a m/z ratio for each compound (Philp, 2004 ; Wang and Stout, 2007) using this selective ion mass spectrometry monitoring technique. Furthermore, this was supported by the confirmation of alkanes and isoprenoids by comparison with the NIST library. This was achieved by conducting a comprehensive search against a reference database of compound mass spectra using the NIST library associated with the Agilent mass spectrometry software. This resulted in positive confirmation of the fragmentogram for the n-alkanes: dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) and the isoprenoids: iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in kerosene oil. A high degree of confidence can be attributed to the correct chemical identification of each compound based on the percentage fit and reverse fit which was found to be greater than 80%. Evidence of these findings is given in sections 3.10 and 3.11 of Chapter 3. Typically 80% or greater is recognised as a strong correlation between the reference compound and the unknown compound.

One of the most important concepts emerging from the research findings presented in Chapter 4 is that the primary mechanism of alteration of kerosene was through evaporation. Reflecting on these findings, it is useful to draw upon the observations presented by Wang and Fingas, (2006a) that the natural attenuation pathway of petroleum releases in soil is predominantly driven by evaporation. One of the limitations of this study and a potential for future research is to develop a laboratory based evaporation study using a variety of different soil matrix types which were found in the research. An understanding of the behavior of the kerosene oil following release into different soil matrix types would improve the performance of the age estimation model. Different soil matrices would affect the rate of weathering of the kerosene oil and would likely have an effect on the transport of the chemical components of the oil (Kaplan et al., 1996)

The weathering experiment of kerosene demonstrated that as evaporation progressed, the mainly lighter n-alkanes were removed first, and eventually the isoprenoids were reduced. This followed the progressive stages outlined by Kaplan et al., (1997). The importance of this process of evaporation is emphasised by the findings in section 4.2 of Chapter 4. This study was conducted by artificially evaporating kerosene oil in a laboratory setting and examined the relationship and depletion of the n-alkanes dodecane (nC_{12}), tridecane (nC_{13}), tetradecane (nC_{14}) and pentadecane (nC_{15}) compared to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil. Prior to analysis, a reference crude oil was analysed and the chromatogram was assessed for suitability. One factor, which has been identified as being important in correlation studies, is the stability of chromatographic resolution of the n-alkanes to isoprenoids and that variations are not due to differences in analytical measurement. This was achieved by ensuring a resolution between heptadecane (nC_{17}) and pristane in the reference crude oil of 1.50 but in fact the resolution was far superior to that and gave a relative standard deviation (RSD) of less than 2%. This confirms the idea that effective separation of the compounds of interest in kerosene was achievable. It was concluded, therefore, that over time it is possible to monitor the depletion of n-alkanes compared to isoprenoids as they are chromatographically separated on the gas chromatographic system.

As discussed in section 4.2 of Chapter 4, the evaporation experiment reduced 4ml portions of neat kerosene oil to 75%, 50% and 25% remaining of their initial volume. It is important, however, to note that this laboratory based evaporation experiment was not conducted in soil nor was the kerosene oil subjected to microbial action. The findings should be taken as an indication of the likely change in the composition of the neat kerosene due to evaporation. This experiment was performed in triplicate to establish an acceptable degree of repeatability. The peak area of each n-alkane and isoprenoid was measured at each evaporation level in triplicate and the RSD was calculated. The percentage RSD was found to be less than 2%. At this level of precision a high degree of confidence can be attributed to the evaporation experiment and analysis. Reflecting on these findings, it is was necessary to demonstrate the laboratory based evaporation profile obtained from the study presented in Chapter 4. This was achieved with the use of a histogram which clearly demonstrates that the n-alkanes in kerosene are preferentially depleting in a progressive order from octane (nC_8) to hexadecane (nC_{16}) as the evaporation level progresses from neat to the 25% of initial volume. Examples of this can be seen from the histogram of the normalised n-alkane in kerosene oil at the different evaporation levels in Figure 6.1.

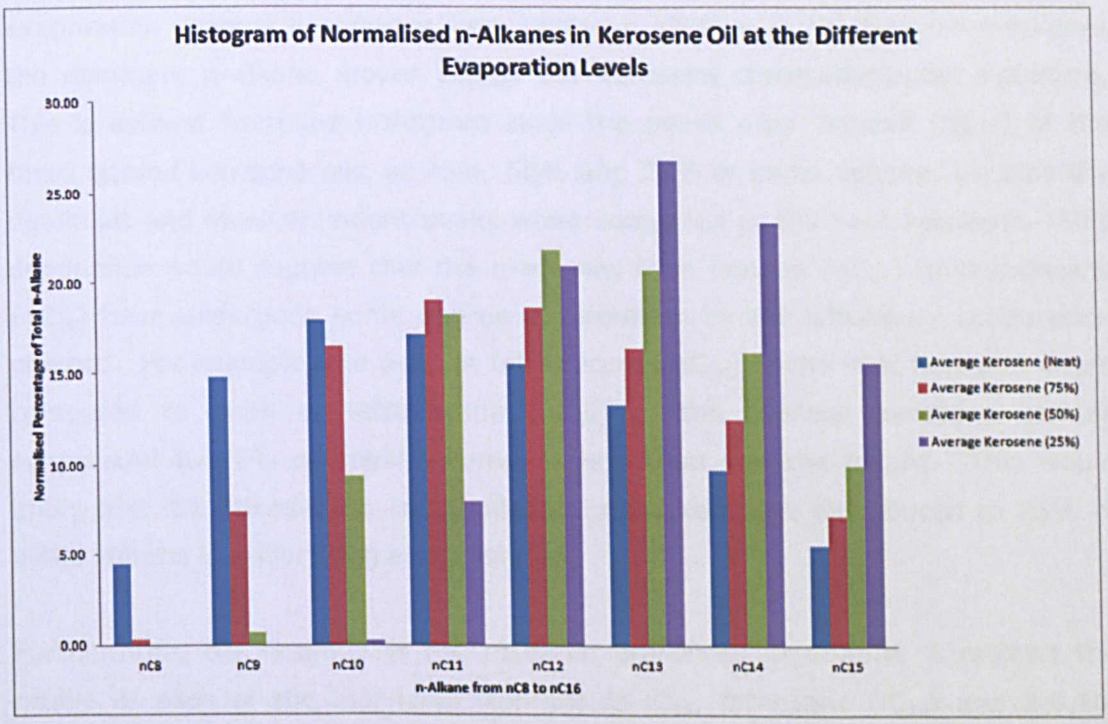


Figure 6.1 Histogram of Normalised n-Alkanes in Kerosene Oil at the Different Evaporation Levels

The findings, when compared to the neat unaltered kerosene oil, can be summarised as follows:

- The neat kerosene had a dominant peak at decane (nC_{10}) having a full range of n-alkanes from octane (nC_8) to hexadecane (nC_{16}). This is supported by evidence presented in Chapter 3 which confirms this profile to be characteristic of fresh unaltered kerosene.

- Kerosene reduced by evaporation to 75% of its initial volume has a dominant peak at undecane (nC_{11}) and is showing reduction of the octane (nC_8) to decane (nC_{10}) n-alkanes.
- Kerosene reduced by evaporation to 50% of its initial volume has a dominant peak at dodecane (nC_{12}) and is showing reduction of the octane (nC_8) to decane (nC_{10}) n-alkanes.
- Kerosene reduced by evaporation to 25% of its initial volume has a dominant peak at tridecane (nC_{13}) and is showing reduction of the octane (nC_8) to nonane (nC_{11}) n-alkanes.

These findings are consistent with the finding that suggest that the n-alkanes series, within kerosene, are depleting in a step wise progressive order according to volatility and vapour pressure as described by Kaplan et al.,(1997). As the evaporation process progresses from lighter n-alkanes to the heavier n-alkanes the dominant n-alkane moves across the kerosene chromatographic signature. This is evident from the histogram since the peaks after nonane (nC_{11}) of the three altered kerosene oils, at 75%, 50% and 25% of initial volume, become the dominant and more abundant peaks when compared to the neat kerosene. This dominance would suggest that the n-alkanes from nonane (nC_{11}) to hexadecane (nC_{16}) have undergone some degree of alteration by the laboratory evaporation process. For example, the peak at tetradecane (nC_{14}) in the neat kerosene when compared to peak at tetradecane (nC_{14}) in the average kerosene sample evaporated to 75% of initial volume, is less than half the height. This would imply that the tetradecane (nC_{14}) alkane in the kerosene oil reduced to 25% of initial volume is undergoing alteration.

Furthermore, the findings of the research presented in Chapter 4 reviews the profile of each of the individual isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) over the different evaporation levels. This demonstrates that the isoprenoids have not altered significantly through the evaporation levels. This is a crucial point and provides evidence that the R^2 findings, presented in Chapter 4, demonstrate that the depletion of the n-alkanes over the isoprenoids is linear and preferential. The relationship between the n-alkane and the isoprenoids was evaluated using a box and whisker plot study and ANOVA. A tight distribution of data was observed demonstrating at each of the evaporation levels a very low deviation within each group. This was validated by a very low percentage relative standard deviation of typically 2%. Conversely the distance between each box and whisker plot, along with the ANOVA results showed that there is a significant difference between the groups. The f-value was significantly lower than the f-critical value. This is to be expected as the difference can be attributed to the preferential depletion of the n-alkane in preference to the associated isoprenoids.

According to the concept presented by Stout et al., (2002a) and Christensen and Larsen (1993), the disparity in the rate of depletion among hydrocarbon classes is due to the isoprenoids degrading more slowly than comparable n-alkanes. This concept forms the basis for the method described by Christensen and Larsen (1993), allowing for the use of the heptadecane (nC_{17}) : pristane ratio to age/date middle distillates. In a similar way to research undertaken by Christensen and Larsen (1993) the rate of depletion of the n-alkanes over isoprenoids in kerosene were reviewed. Within the findings, evidence for this can be found to show that the ratio of the n-alkane to the isoprenoid obtained for dodecane (nC_{12}) : iC_{14}, tridecane (nC_{13}) : farnesane (iC_{15}) and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) from each of the evaporation levels and the neat kerosene oil demonstrated preferential depletion of the n-alkane over the corresponding isoprenoid. A linear regression study was undertaken and the linear regression plot for each of the n-alkane to isoprenoid ratios demonstrated a linear relationship with correlation coefficient (R^2) values of:

- Dodecane (nC_{12}) : iC_{14} v. Percentage Evaporated R^2 = 0.9808
- Tridecane (nC_{13}) : farnesane (iC_{15}) v. Percentage Evaporated R^2 = 0.9729
- Tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) v. Percentage Evaporated R^2 = 0.9900

These findings are crucial as they underpin the objective of this research question:

> *To evaluate the preferential depletion of the n-alkanes dodecane (nC_{12}), tridecane (nC_{13},), tetradecane (nC_{14}) and pentadecane (nC_{15}) to the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil, by conducting a series of artificial weathering experiments (Chapter 4).*

The linear relationship found in section between the ratio of n-alkane to isoprenoid versus each of the percentage of initial volume after evaporation clearly demonstrates a preferential depletion of the n-alkane over the isoprenoid.

It is however important to caveat this statement with a caution as the experiment was based on the depletion of n-alkanes and isoprenoids in a neat kerosene oil. While this does demonstrates preferential depletion of the n-alkane over the isoprenoids the effects associated with different matrix types would need to be evaluated.

6.3 Research Question – Development of Database

The next two research questions drove the research to develop a database of chromatograms and associated metadata obtained from the analysis of kerosene spill samples (soil and water) received for analysis between 2009 and 2011 at Jones Environmental Forensics Limited laboratory. These samples were submitted for analysis by environmental consultants working on domestic home heating oil releases in Ireland and the United Kingdom and the research findings are presented in Chapter 5. However, within the course of the research the requirement to develop an analytical method for the rapid extraction of isoprenoid and n-alkane content (Peak areas / peak heights) from chromatographic data obtained from the analysis of each sample was necessary due to the large number of chromatograms required for interpretation and review. The guide subsequently generated is presented in Appendix 2.

It is worth noting that over 6,100 chromatograms were reviewed at the start of this study. However, before any data were collected it was necessary to scrutinise of all the 6,100 chromatograms to identify suitable examples which contained the characteristic profile of kerosene. Furthermore, this scrutiny found that not all of the 6,100 chromatograms were suitable for inclusion within the study as they either contained profiles which did not reflect a kerosene contaminant or were considered to be free from contamination. During the screening of the 6,100 chromatograms, many of the profiles reflected contamination which was characteristic of a wide variety of petrogenic and pyrogenic contaminant sources. Profiles such as degraded diesels, lubrication oil, heavy fuel oils, gas oils, mineral insulating oils, cable oils, creosote, coal tar, PAHs, bitumen, naturally occurring humic acids and many more were found, all were excluded from the study. However, within the course of the chromatogram review, 473 chromatograms were found to contain the characteristic profile of kerosene at different stages of alteration. It was clearly apparent, from these fingerprints, that many of the selected chromatograms were at different stages of weathering or biodegradation as described by Wang and Fingas, 2006a; Oudijk, 2009; Chapelle (2001); Kaplan et al., 1996; and Kaplan and Galperin (1996) which was discussed further in Chapter 2.

The next challenge was to gather the peak area data from each of the 473 chromatograms for the n-alkanes and isoprenoids. This created a further challenge as it was necessary to develop a rapid and accurate data collection procedure. A step by step guide to the correct integration and selection of the n-alkanes and isoprenoids was developed (Appendix 2) which answered the second research question:

> *To develop an analytical method for the rapid extraction of isoprenoids and n-alkanes content from chromatographic data.*

The purpose of this guide was to allow laboratory technicians in the future to reproduce this data collection process when the kerosene age estimation model is brought to commercialization. This is a crucial and critical procedure as the correct integration of the n-alkanes and isoprenoids is necessary to determine the ratio of n-alkane to isoprenoid used in the final age estimation model.

Once all of the n-alkane and isoprenoid chromatographic data was obtained from the 473 chromatograms, for the n-alkanes: dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) and the isoprenoids: IC_{14}, farnesane (IC_{15}) and 2,6,10-trimethyl-tridecane (IC_{16}); it was necessary to include other metadata which was available within the Jones Environmental laboratory final report for each consultant. This data gathering exercise was described in Chapter 3. It is important to note that anonymity of participants, due to confidentiality agreements with each consultancy, was required. Written permission to use the data within this study was obtained upon engaging with each consultancy firm and the restrictions of use of the data are given in Chapter 3. Following a number of discussions with each of the environmental consultancy firms paragraph 3.14.2 in Chapter 3 outlined the reasons given for the exclusion of samples from the 473 samples collected. This resulted in the dataset condensed down to 43 samples.

Furthermore, each consultant was contacted and asked to provide information about the conditions pertaining to the site from where the samples were taken. Chapter 3 details the list of information obtained from a review of the chromatograms, final laboratory report and discussions with consultancy firms. From these discussions it was evident that the model would be of benefit to each consultancy firm. However, it would be very important to discuss with each consultant the appropriate samples techniques used. Additional analysis of biomarkers or the alkylated polycyclic aromatic hydrocarbons by GC-MS may add additional diagnostic ratios which could be used with the model. However, after a number of conversations with different consultant project managers it was clear that commercial costs associated with winning these projects would have an impact on limiting any additional analysis. Another important consideration is that every consultancy firm have different standard operating procedures (SOP) associated with sampling, and each project also presents different sampling challenges. Therefore, it is important and critical that a complete site history and sampling work plan is reviewed when using the model.

This allowed for the generation of the kerosene data base and answered the research question:

> *Develop a database of chromatograms and associated metadata obtained from the analysis of kerosene spill samples (soil and water) received for analysis between 2009 and 2011 at Jones Environmental Forensics Limited laboratory. These samples have been submitted for analysis by environmental consultants working on domestic home heating oil releases in Ireland and the United Kingdom.*

6.4 Research Question – Environmental Influencing Factors

The fourth research question required an analysis of the information gathered in the database.

> *Analyse the database of isoprenoid and n-alkane data of kerosene spill samples (soil), in order to evaluate the environmental factors (weathering and biodegradation) affecting isoprenoid and n-alkane ratios in post-spill kerosene in soil in order to accurately date a spill event.*

The physical and chemical characteristics of the soil type found at the sampling locations can influence the retention, transformation and movement of pollutants through the soil (USEPA, 1992). Once kerosene has been released into the soil its fate can be affected by the dynamics of moisture conditions in the subsurface as a result of rainfall, irrigation, and fluctuation of the ground water level (Dror, Gerstl, Prost et al., 2002). As discussed in Chapter 2, in order to perform any environmental forensic study, it is always important to understand the soil conditions pertaining to the site. Dry conditions in soil or stagnant conditions in groundwater will suppress the rate of alkane attenuation, whereas rapid water flow will bring oxygen to the site and enhance biodegradation (Atlas and Bartha, 1992; Kaplan et al., 1997). It would be an advantage to speak to environmental consultants about the physical and chemical characteristics at project sites to fully understand the constraints which can be applied to the use of the model.

In Chapter 5, the soil type described by each of the environmental consultants was broken down into the following categories: 37% clay, 23% sandy gravely clay, 23% clay silt, 7% made ground fill, 5% topsoil, and 5% clay with peat. The majority of the soil types had the presence of clay. The percentage natural moisture content (%NMC) for the different soil categories fell between 9.25% and 71.82% for the 43 samples used to evaluate the selection of the most appropriate age estimation model. The percentage natural moisture content was further broken down in Chapter 5 into the different soil type categories which were obtained from each of the environmental consultants. It was observed that clay had the widest range of moisture content.

As described in Chapter 2, the rate of alteration is predominantly influenced by the availability of oxygen and nutrients. Therefore the type of soil impacted and the moisture content may influence the rate of natural attenuation. Soil texture such as the more permeable coarse-grained soil allows for the replenishment of oxygen and, therefore enhances biodegradation (Oudijk, 2009). The lack of oxygen can limit the aerobic microbes' ability to degrade kerosene releases through natural attenuation (Atlas and Bartha, 1992; Oudijk, 2009). The moisture present in the soil can influence this rate of degradation and a low level of moisture normally exhibits a decreased biodegradation rate (Oudijk, 2009). As stated in Chapter 5 these findings embody the idea that the key mechanisms of alteration found in this study are a combination of volatilization and biodegradation. Reflecting on these findings, it is useful to draw upon the knowledge that proximity to the ground surface allows for greater oxygen abundance and therefore greater volatilisation (Oudijk, 2009). It was therefore important to establish a minimum depth as samples retrieved from near the surface were found to exhibit a faster alteration, and therefore these samples were excluded from the model. The findings in Chapter 3 resulted in a soil depth ranging from 0.4m to 1.9m. A linear regression trend line was calculated for the data and the coefficient of determination (R^2) was used as a means of determining the point at which the minimum depth and concentration was achieved to produce a R^2 of greater than 0.7. A conservative cut point for R^2 of 0.7 or higher was recommended by Wiens, Dale and Boyce et al., (2008). 58% of the soils used within this research were between 0.4m and 0.5m, with the remaining 42% between 0.6m and 1.9m.

The minimum concentration of 1,000 mg/kg was determined within the findings, evidence for this can be found in Chapter 3. Closely linked with this is the principle that at higher concentration, a better chromatogram for interpretation can be obtained. This minimum concentration makes it easy to identify and gather the chromatographic data of peak height for each of the n-alkanes and isoprenoids due to the abundance of the peaks present. A minimum concentration of extractable petroleum hydrocarbon for kerosene ranged from 1,025 mg/kg to 18,734 mg/kg with the majority (77%) of the samples between 1,000 mg/kg and 7,000mg/kg and the remaining 23% of the samples falling between 7,001 mg/kg and 19,000 mg/kg.

The use of p-values was employed to support the R^2 values findings describe above. Small changes observed in the p-values increased as both depth and concentration increased. A notable change in the p-value was observed at a depth of 0.4m and at a concentration of 1,000 mg/kg. This change represented an improvement in the correlation of the data associated with samples at greater depths (>0.4m) and at greater concentrations. Concentration is an important consideration as the chromatographic profile of samples less than 1,000 mg/kg may be difficult to interpretive.

Unfortunately, other soil chemistry parameters, such as pH, organic matter content, sulphide content, temperature, microbial population assessment and other nutrient analysis (nitrogen and phosphorus), which may influence the rate of alteration following a release of kerosene were not undertaken by Jones Environmental Forensics Ltd on the samples which were submitted for analysis by the environmental consultants. The remediation consultants typically do not go into this level of detail during this type of site investigation as both time and cost are significant factors. Following a private communication with one of the senior consultants whose data was used within this study, Nagle (2012) explained that the consultancy company he works for approaches an initial survey investigation to obtain the following information:

- To map out the contaminant plume on site,
- To carry out a risk assessment to ascertain the severity of the risk to receptors using the source-pathway-receptor model. Such receptors would include buildings and other structures, occupants of property, flora and fauna etc.
- To carry out a desktop risk assessment to ascertain the risk to watercourse, groundwater, nearby well locations etc.
- Justification of proposed remedial works via a risk based approach.

Furthermore, Nagle (2012) explained that the consultancy company he works for are: "*not really concerned with the alteration or breakdown of kerosene within the soils or external influences which may have an impact on same.*" He further explains that it is his company's intention to "*excavate and remove this material from site at the earliest possible opportunity where practical to do so*"

He noted: "*initial survey samples usually consist of samples containing the highest levels of contamination obtained during the survey to highlight the risk associated with such contamination and also clean samples to demonstrate areas that have not been impacted*". Furthermore, Nagel (2012) concluded by saying: "*the cost of the additional analysis (pH, sulphide, temperature, microbial population and nutrients) is not really a factor as in the vast majority of cases as [the consultancy in question] do not need to get involved in the extent of the breakdown or degradation of kerosene within the soil therefore the extra analysis parameters would not be beneficial.*"

6.5 Research Question – Model Development

The second last remaining research question driving this study explores the different types of equations evaluated within Chapter 5. The research question is:

> *Develop an equation equivalent to the Christensen and Larsen (1993), Kaplan, Galperin, Alimi et al., (1996b) equation used for middle distillate, but based on forensic markers of isoprenoids and n-alkanes present in kerosene, which is applicable to kerosene spills (Chapter 6).*

To address this question of development of an equation equivalent to the equation generated by the Christensen and Larsen (1993), Kaplan, Galperin, Alimi et al., (1996b) study, a descriptive statistical review of five model equations was undertaken. The concept of descriptive statistics described in Chapter 5 is simply the numerical procedure or graphical techniques used to organise and describe the characteristics or factors of a given sample (Fisher and Marshall, 2009). The statistics used within this study were primarily concerned with how to summarise and interpret the environmental variables likely to influence the predictive nature of the final model (DeCoster, 1998). These environmental variables include volatilization, biodegradation and chemical alterations of the released kerosene following release into soil, all of which were assessed in Chapter 4 and Chapter 5.

The findings presented in Chapter 4 and 5 would suggest that evaporation, in the short term after a spill, was the primary weathering process affecting the chemical composition of spilled kerosene which was previously described by Wang and Stout (2007) and Wang and Fingas (2006a). However, within Chapter 5, biodegradation was found to be the primary mechanism involved in the alteration of the n-alkanes in preference to the isoprenoids through the natural weathering and the eventual removal of kerosene from the environment found in the samples reviewed within this research (Snape, Harvey, and Ferguson et al., 2005). This hypothesis of biodegradation finds strong support within the findings and will be discussed further within this chapter.

6.5.1 Model Selection and Validation

Within Chapter 5, the reliability of each of the statistical algorithm trend lines was measured by its coefficient of determination R-squared value (R^2). The objective of the statistical trend line analysis in Chapter 5, identified a model that will accurately predict *y* as a function of a set of predictor variables *x* taking into account all of the environmental influences (evaporation and biodegradation) at the spill location. From the kerosene database presented in Chapter 5 each of the ratios for dodecane (nC_{12}) : iC_{14}; tridecane (nC_{13}) : farnesane (iC_{15}); and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release generated an equation and the associated R^2 value for the following regression models:

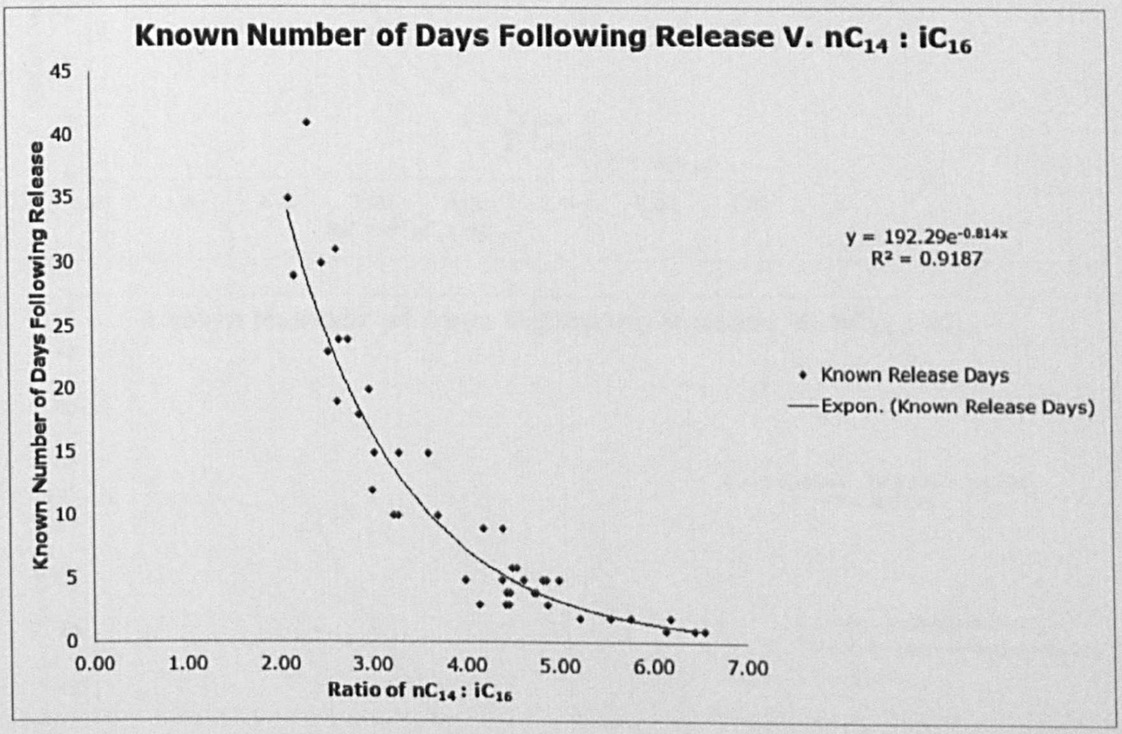
- Linear Regression Model,
- Exponential Regression Model,
- Power Regression Model,
- Logarithmic Regression Model, and
- Polynomial Regression Model.

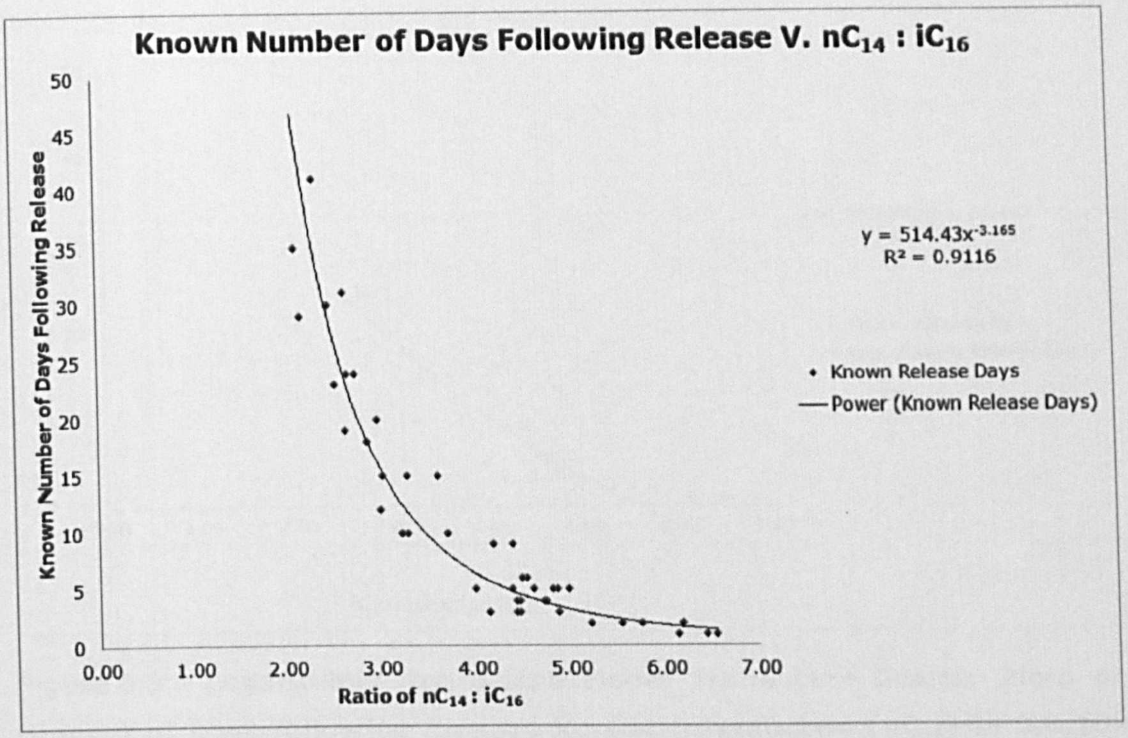
The findings presented in Chapter 5 clearly showed that resulting R^2 value and the linear equation calculated from the ratio of dodecane (nC_{12}) : iC_{14} and tridecane (nC_{13}) : farnesane (iC_{15}) versus the known number of days following release are likely to undergo a greater degree of alteration within the first ten days following release. The scatter plot resulted in a poor R^2 value of 0.3762 for dodecane (nC_{12}) : iC_{14} and 0.68 for tridecane (nC_{13}) : farnesane (iC_{15}) versus the known number of days following release. Reflecting on these findings, it is clear that n-alkanes and isoprenoids up to and including tridecane (nC_{13}) follow the progressive stage of weathering which was described by Kaplan et al., (1997). Furthermore, the ratio of tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release produced a R^2 of 0.7438 following a fit of a linear regression trend line to the dataset. Therefore, it may be argued that the ratio of tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release produced a R^2 value higher than the 0.7 R^2 value recommended by Wiens, Dale and Boyce et al., (2008). They argued that an R^2 value lower than 0.7 would act to disqualify combinations of predictors in a model.

It can be seen from the linear regression scatter plot paragraph 5.5.1 of Chapter 5, of number of days following release versus the ratio of tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) that the data in the first ten days following release does not tend to follow a linear pattern. It is clear the predictive ability of the linear model would not be good for early releases and late releases.

The profile appears to follow a curve, which, may be better represented by an exponential, power or polynomial regression trend line. The findings suggest that evaporation appears to have a greater effect on the lighter n-alkane, (nC_8) to tridecane (nC_{13}) and isoprenoid iC_{14} and farnesane (iC_{15}) which is supported by the progressive stages outlined by Kaplan et al., (1997). In contrast, the findings also suggest that the heavier n-alkanes - tetradecane (nC_{14}) to hexadecane (nC_{16}) and isoprenoid, 2,6,10-trimethyl-tridecane (iC_{16}) appeared to differ with respect to degradability due to their stereochemistry rather than vapour pressure alone. Therefore, the n-alkane, tetradecane (nC_{14}) is likely to be more susceptible to microbial alteration than the isoprenoid, 2,6,10-trimethyl-tridecane (iC_{16}). This corresponds with the observations of Christensen and Larsen (1993). The findings within this research support this observation as it appears to be evident in the trend line that the ratio of tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) produced a good R^2 value. This would suggest that evaporation may be considered as one of the influencing mechanisms of alteration of the ratio but may not be the main factor. Christensen and Larsen (1993) have suggested that microbial degradation is the primary mechanism for the preferential removal of the n-alkanes in preference to the branched isoprenoids in the heptadecane (nC_{17}) : pristane ratio used in their study. This forms the basis for their model where the isoprenoid gradually becomes more dominant as the corresponding n-alkane is degraded over time. Reflecting on this concept, the findings suggest that microbial degradation is the main mechanism of alteration.

Similarly to the Linear regression model, the ratios of dodecane (nC_{12}) : iC_{14}; tridecane (nC_{13}) : farnesane (iC_{15}); and tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release were plotted. Exponential, power, logarithmic and polynomial regression equation models were fitted to the data to determine the R^2 value for the fitted trend line for each type of equation. Once again, it was clear that the ratios of dodecane (nC_{12}) : iC_{14} and tridecane (nC_{13}) : farnesane (iC_{15}) were affected by weathering as the R^2 value were outside the acceptable range. This implies that the use of these ratios would not result in a good predictive model, and therefore, these were subsequently excluded from the model assessment process. The scatter plot in Figure 6.2 represents the exponential, power and polynomial trend lines which were fitted to the data resulting in good R^2 values. The findings presented in Chapter 5 for the logarithmic regression model represented a good R^2 value of 0.8354 (Figure 6.3). However, the fitting of the logarithmic data may present a problem when estimating predicted values. This will be evaluated and discussed later within this chapter.





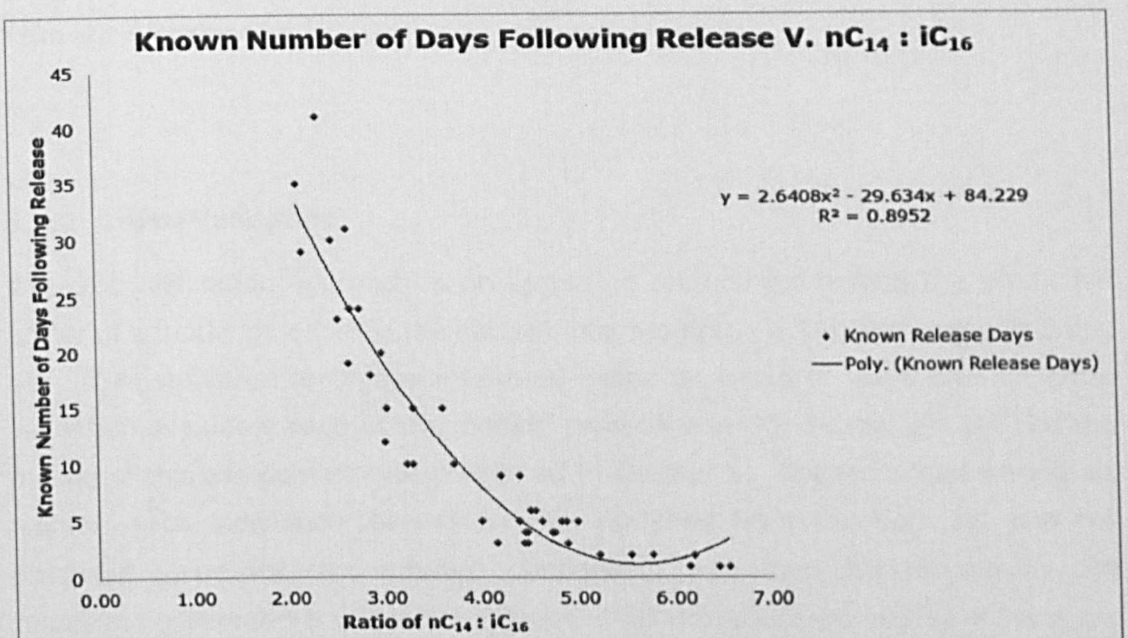


Figure 6.2 Exponential, Power and Polynomial Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

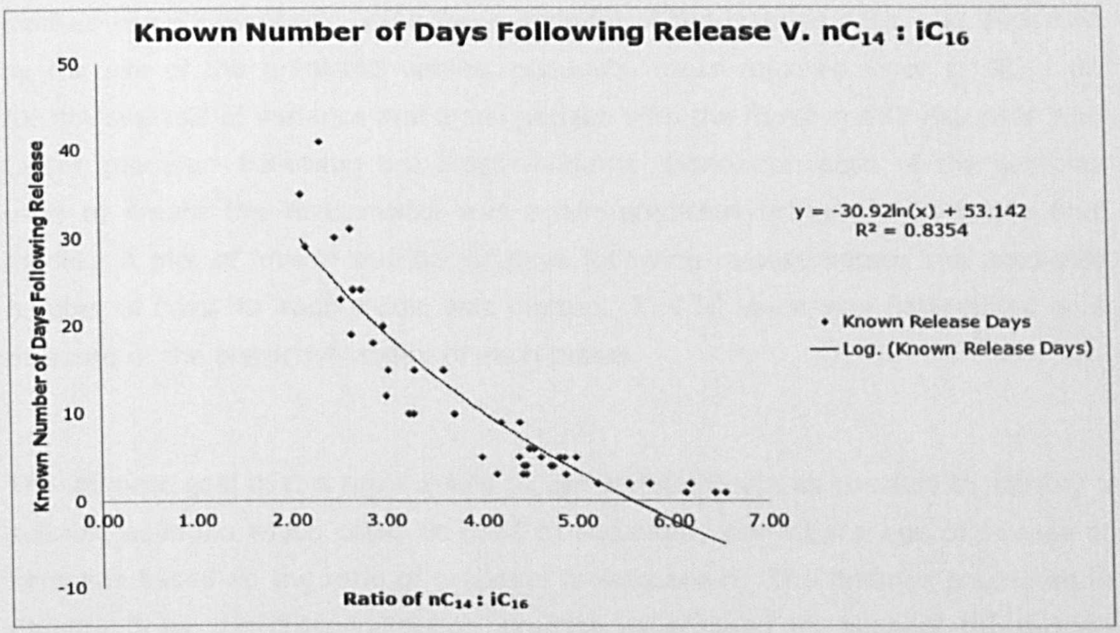


Figure 6.3 Logarithmic Regression Model Trend Line Scatter Plots of Number of Days following Release Versus Tetradecane (nC_{14}) to 2,6,10-Trimethyl-tridecane (iC_{16})

6.5.2 Cross-Validation

The cross-validation approach is an appealing method for testing the predictive ability of a model by dividing the dataset into two sets - a 'test set' and a 'training set'. This statistical technique measured based on *k*-fold or leave-one out cross validation evaluated each of the models' predictive ability on the test set and the finding of this assessment was presented in Chapter 5. The technique worked as follows: each individual observation was removed from the data set and the predicted value for this omitted observation was then determined by the remaining observations within the dataset. This procedure worked by dividing the dataset into two sets called the 'test set' and the 'training set' (called leave-one-out cross-validation) where all of the data were left out at least once. The residual was determined as the difference between the number of days following release (Release Days) and predicted value by the model. In this way, various candidate models were tested so that the best choice can be selected on the basis of predictive performance with the validation data. This division process allowed for each model to be assessed using as much data as possible to obtain an estimate but allowing the model to test data which was excluded from the model-building process. The ability of each model to accurately predict each of the omitted sample numbers, using the remainder of the training data, was assessed by the use of the predicted values, residuals, mean squared error (MSE) - an ANOVA analysis of variance and a comparison with the Hurst and Christensen and Larsen models. Following the cross-validation evaluation each of the samples used to create the final model was again predicted using the complete final model. A plot of known number of days following release versus the predicted number of days for each model was plotted. The R^2 value was determined as a measure of the predictive ability of each model.

The ultimate goal of this project was to use as much data as possible to identify a suitable equation which could be used to accurately predict the age of release of kerosene based on the ratio of n-alkane to isoprenoid. The findings presented in Chapter 5 for the cross-validation exercise determined for each of the models (exponential, linear, power, logarithmic and polynomial) the predicted estimate, the residual and the mean squared error. The residual was calculated as being the difference between the predicted estimates for each sample and the known number of days since release. The squared error was calculated from the absolute value of the residual for each determination. The mean of the squared error for each model was reported as the mean square error (MSE) and could be used to compare the different models. The findings of the cross validation was undertaken in Chapter 5 for each of the different models under review within this study. Table 6.2 summaries these findings by reporting the MSE for each of the regression models. It is clear that the Logarithmic regression model can be eliminated as a potential model due to its high MSE when compared to the other models. To establish a benchmark, the cross validation exercise was applied to the data used to generate the Hurst, and Christensen and Larsen models. The findings were presented within Chapter 5 where the MSE for these models was lower than that determined for the kerosene age estimation models.

Table 6.2 Summary of MSE for Exponential, Linear, Power, Logarithmic, Polynomial Regression Models

Model	MSE
Exponential Regression Model	12
Linear Regression Model	32
Power Regression Model	18

Model	MSE
Logarithmic Regression Model	614
Polynomial Regression Model	13
Christensen and Larsen Linear Regression Model	5
Hurst Linear Regression Model	3

6.5.3 Cross Validation Comparison and Model Statistical Evaluation

The cross validation findings for the Hurst linear regression model in Chapter 5 found the model reported a MSE of three. Using this MSE as a benchmark, a cross validation exercise was undertaken on each of the individual models (Exponential, Linear, Power and Polynomial) by iteratively removing outliers and recalculating the model until each model achieved a MSE of three. Chapter 5 presents a series of plots of the known age versus the predicted age determined for each of the models. From these plots the R^2 value was determined for both the initial cross validation exercise and the adjusted cross validation exercise. An ANOVA statistical evaluation was undertaken for each model before and after the elimination of outliers to determine the *f*-test value for both sets of data. The ANOVA is used as a measure of different sources of error between the predicted values and the actual values (EA, 2012). The *f*-test compares the variance of the two data sets, the actual known release days against the predicted days. If the calculated *f*-value is less than the *f*-critical in the ANOVA statistical test then there is no significant statistical difference between the two data sets (EA, 2012). The *p*-value represents the probability that variation between the known release date and the predicted value may have occurred. The percentage relative standard deviation as a measure of precision was calculated from the absolute values of the residual. The following summarises the findings reported in Chapter 5 for each of the models.

- The Exponential Regression Model removed 8 outliers from the total dataset. The model had a prediction range of 1 to 29 days. The maximum residual was found to be 3 days with a minimum of -4 days. The initial R^2 value for the predictive ability of the model was found to be 0.9074 and following removal of outliers this improved the R^2 value to 0.9278. The ANOVA *p*-value moved from 8.47E-23 to 2.11E-20. The relative standard deviation reduced from 87% to 86% which demonstrates a minor improvement in the precision of the model.

- The Linear Regression Model removed 22 outliers with a model prediction range of 2 to 24 days. The model had a maximum of 3 days and minimum residual of -4 days. The initial R^2 value for the predictive ability of the model was found to be 0.7113 and following removal of outliers this improved to a R^2 value of 0.9379. The ANOVA p-value moved from 1.27E-12 to 6.38E-13. The relative standard deviation reduced from 83% to 68% demonstrating an improvement in the precision of the model.
- The Power Regression Model removed 7 outliers and had a model prediction range of 1 to 40 days. The model had a maximum and minimum residual of +/-4 days. The initial R^2 value for the predictive ability of the model was found to be 0.8623 and following removal of outliers this improved to 0.9534. The ANOVA p-value moved from 2.76E-19 to 6.88E-25. The relative standard deviation reduced from 94% to 91% demonstrating and improvement in precision.
- The Polynomial Regression Model removed 12 outliers and had a model prediction range of 1 to 35 days. The model had a maximum and minimum residual of +/-4 days. The initial R^2 value for the predictive ability of the model was found to be 0.8823 and following removal of outliers this R^2 value improved to 0.9557. The ANOVA p-value moved from 1.18E-20 to 3.53E-21. The relative standard deviation reduced from 88% to 103% demonstrating a slight disimprovement in precision.

For all of the models, the f-test ANOVA value was less than the f-critical ANOVA value which suggested that there was no significant statistical variation between the known release date and the predicted release date for each model. In all cases the f-test value improved for the models with the eliminated sample numbers. The measure of precision, the percentage relative standard deviation, for each model was calculated before and after elimination of sample numbers and in all cases with the exception of the polynomial model the precision significantly improved. The findings are presented in Chapter 5 where the models were compared to the Christensen and Larsen linear regression model (%RSD = 71%) and the Hurst linear regression model (%RSD = 85%).

The power and the exponential regression models have the least number of sample numbers eliminated from the revisited cross validation exercise (7 and 8 sample respectively) making these two models the best choice when compared to the Linear (22 sample numbers eliminated) and polynomial (12 sample numbers eliminated) models. It is important to include as many sample numbers as possible within the final model to ensure that the model is as robust as possible in order to reflect the typical environmental conditions where the model will be applied. This would suggest that the power and exponential regression models are the best choice on these criteria as they include the most sample numbers.

The power regression model was found to have the widest model prediction range of 1 to 40 days with the linear regression model demonstrating the lowest range of 2 to 24 days. The exponential and polynomial regression models had similar model prediction ranges of 1 to 29 days and 1 to 35 days respectively. This would suggest that the power regression model is the best choice as it would be important to have as wide a predictive range as possible for the model.

The exponential and linear models were both found to have the best minimum (-3 days) and maximum residual of +4 days. The power and polynomial models both had a minimum and maximum of +/-4 days.

A plot of the known number of days following release compared to the predicted number of days was used to calculate an R^2 value for each of the models. This was used as a measure of how well the model predicted the known release date for each sample number. The findings within Chapter 5 suggested that the power and polynomial regression models had the highest R^2 value of 0.9229 and 0.9652 respectively when compared to the other two models making theses model the best choice based on this criteria.

The scatter plots of known number of days following release versus the predicted release date presented in Chapter 5 for each of the models before the sample numbers were eliminated demonstrated that the models were more likely to accurately predict a release within the first ten days and as time progressed this became less accurate.

In conclusion the power model appears to be the most appropriate choice of model to be used to age estimate the release of kerosene.

6.5.4 Environmental Factors Affecting the Elimination of Sample Numbers from the Selected Power Regression Model

Factors, which contribute to the justification of the removal of outliers in the development of the power model, were found to be dependent on a variety of parameters regarding soil type, depth, concentration and moisture content. The percentage natural moisture content (%NMC) for the different soil categories is described in Chapter 5. It ranged from 9.25% and 71.82% for the 43 samples used in the evaluation for the selection of the most appropriate model. Each of the eliminated sample numbers will now be examined.

6.5.4.1 Elimination of Sample Numbers 387 and 452 from the Power Regression Model

Both 387 and 453 sample numbers had been described as soil types containing peat. Huat, Kazemian, Prasad et al. (2011) have described peat as a soft soil whose composition can account for at least 65% of organic matter with a very high water content. Oudijk, (2009) described both high water and high organic matter content as factors which promote aggressive natural degradation. For the Power Regression Model the sample numbers 387 and 452 were found to overestimate the age of release; 66 days instead of 29 days for sample number 387, and 63 days instead of 35 days for sample number 452. This over prediction would suggest that the samples had undergone a higher degree of alteration when compared to the other predicted sample number estimations included within the model. Within the findings the %NMC for sample numbers 387 and 452 was found to be 68.65% and 65.46% respectively. As described in Chapter 2 by Oudijk, (2009) these conditions would promote the mechanism of biodegradation. Within the data set, these were the only two samples found to contain peat. It is worthy of note that these two sample numbers were eliminated from the other models assessed within this study, see findings in Chapter 5. The depths from which these two samples were taken namely, 0.60-0.70m (387) and 0.4m (452) do not appear to influence the predicted findings since the depths fall within the expected range for the model which was found to be 0.4m to 1.9m. The concentrations - 4,512 mg/kg (387) and 1,227 mg/kg (452) - do not appear to influence the elimination of the two samples as these concentrations fall within the range (1,000 mg/kg – 7,000mg/kg) of the majority (77%) of the samples used within the study.

6.5.4.2 Elimination of Sample Number 275 and 240 from the Power Regression Model

The next three eliminated sample numbers were 275 and 240. The three sample numbers were described as sandy gravelly clay with %NMC of 55.65% and 49.34% respectively, which is significantly higher than the average %NMC for the seven other samples of this soil type, which was 22.45%. Outlined in Chapter 2, Oudijk (2009) suggested that a coarser-grained soil type tends to allow for the replenishment of oxygen, which will promote the presence of microbes and therefore enhance the degradation process. The power model was found to overestimate the age of release for sample number 275 by 5 days - with a prediction of 24 days compared to the known release of 19 days. Furthermore for sample number 240, the model overestimated by 4 days - with a prediction of 16 days compared to a known release of 12 days. The overestimates may be accounted for by increased microbial biodegradation linked to the high level of %NMC for this soil type. The depths from which these two samples were taken, 0.90 – 1.0m (275) and 1.0m (240) were within the model's depth limits of 0.4m to 1.9m. The concentrations for the two samples were determined as 13,735 mg/kg for sample number 275 and 1,614 mg/kg for sample number 240. Stout, Uhler, McCarthy et al., (2002b) suggested that a maximum concentration may be warranted because soils that are completely saturated with petroleum may be toxic to microbes, which would lessen the rate of biodegradation. For these two samples it would appear that the concentration levels found in the samples may not have affected the microbial population at these sample locations and result in an over estimation of the age of release.

6.5.4.3 Elimination of Sample Number 77 and 258 from the Power Regression Model

The eliminated sample numbers 77 and 258 had soil descriptions of clay with a %NMC of 56.94% and 66.57% respectively, which is significantly higher than the average %NMC for the eleven other samples of this soil type, which was 26%. Sample numbers 77 had a known release date of 15 days where the model produced a prediction of 9 days. Sample number 258 had a known release of 31 days with a predicted value of 23 days. The concentrations were determined as 2,969 mg/kg (258) and 8,268 mg/kg (77). Sample numbers 77 and 258 were both taken from a depth of 0.4m. The nature of the soil in these samples would suggest that microbial degradation may be inhibited and result in an under estimation of the age of release.

6.6 Research Question – Model Development

In conclusion, the evaluation of the cross validation exercise would suggest that the power regression model is the most suitable model to be used for the age estimation of kerosene releases (Figure 6.4). The equation to calculate the age of release of kerosene was determined to be $y = 686.88(nC_{14} : iC_{16})^{-3.357}$ +/- 4 days.

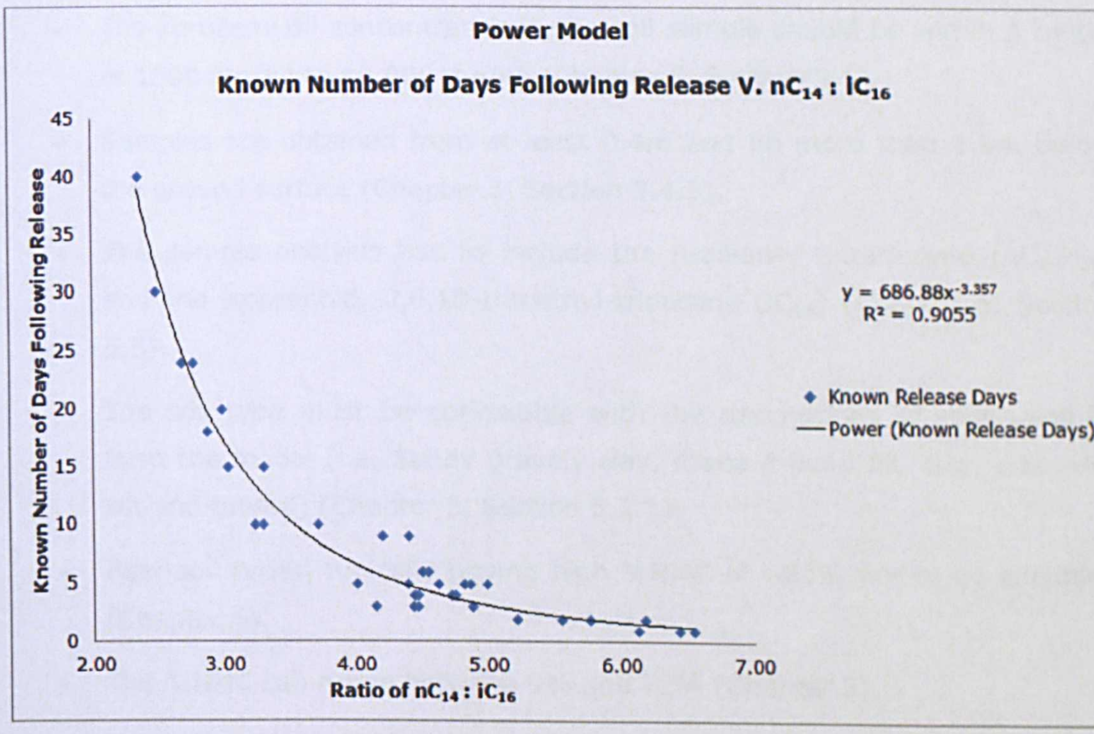


Figure 6.4 Power Regression Model

Having reviewed and discussed all of the factors which hinder or facilitate the use of the model within this chapter it is useful to reflect on how these would relate to its use in predicting kerosene releases. It is important to draw on all of the findings of this research and elicit criteria for its use. Furthermore, this fulfills the final research question:

Elicit criteria constraining the conditions within which the kerosene spill ageing equation can be relied upon.

It is important to set out a series of conditions under which the use of the power regression model in the estimation of age of a kerosene release is valid. The following conditions must be met before using the model.

- This model is only applicable in samples where the only contaminant present is kerosene (Chapter 4). The presence of high levels of another contamination source such as diesel, which also contains the markers tetradecane (nC_{14}) and 2,6,10-Trimethyltridecane (iC_{16}), could possibly have an adverse impact on the diagnostic ratio that has been derived leading to an erroneous estimate of age.
- The kerosene oil concentration in the soil sample should be within a range of 1000 mg/kg to 19,000 mg/kg (Chapter 5, Section 5.1).
- Samples are obtained from at least 0.4m and no more than 1.9m below the ground surface (Chapter 3, Section 3.4.3).
- The sample analysis has to include the n-alkane, tetradecane ($nC_{14}H_{30}$) and the isoprenoid, 2,6,10-trimethyl-tridecane (iC_{16}) (Chapter 5, Section 5.5).
- The soil type must be compatible with the descriptions of soils used to form the model (i.e. Sandy gravely clay, made ground fill, clay, clay with silt and topsoil) (Chapter 5, Section 5.2.1).
- Peat soil types, typically having high %NMC of >65% are to be excluded (Chapter 6).
- The %NMC can range between 9% and 72% (Chapter 5).
- The release is sudden (Chapter 2).
- The sample is obtained before remediation action is commenced at the spill location.

6.7 Further Power Model Validation

The data presented in Table 6.3 became available at the end of the research; this data can be used to further validate the Power Kerosene age estimation model.

Table 6.3 Power Model Validation Additional Data

No.	Estimated Release Date	Sample Collection Date	Known Number of Days Following Release	EPH Concentration mg/kg	%NMC	Depth (m)	Soil Type Description Given by Consultant
474	13/06/11	14/06/11	1	3,391	9.1	0.45	Gravel and Fill Material
475	13/06/11	14/06/11	1	1,881	9.6	0.4	Gravel and Fill Material
476	27/1/12	6/02/12	10	1,319	31.9	0.5	Clay / Sandy Gravel
477	2/05/12	10/05/12	8	8,807	11.2	0.7	Clay

For each of the samples 474 to 477 the tetradecane (nC_{14}) and 2,6,10-Trimethyltridecane (iC_{16}) was determined from the chromatogram which was extracted from the soils submitted to Jones Environmental Laboratory. This was done using the process described in Appendix 1 and Appendix 2.

Table 6.4 Power Age Estimation Model Prediction

No.	Known Number of Days Following Release	nC14 : iC16 Ratio	Model Prediction	Residual
474	1	5.50	2	1
475	1	5.14	3	2
476	10	3.20	14	3
477	8	4.33	5	-3

Sample 474 and 475 were over estimated by the power model, this overestimation is likely to be influenced by the soil type which was described by the consultant as "gravel and fill material". The kerosene oil in this environment is likely to have an enhanced level of degradation due to the oils rapid migration through this environment. Similarly the overestimation observed in sample 476 may be due to the %NMC (31.9%) in clay / gravel environment. Finally the underestimation in sample 477 may be due to a combination of a low %NMC found in the clay soil type which is an environment where microbial degradation may be inhibited. Similar findings were described for the samples omitted from the model in section 6.5.4 within this chapter.

7.0 Chapter 7 Conclusion and Future Work

This final chapter reflects on the outcomes of this research. This chapter will review how each of the objectives was achieved and how the selected model has contributed to the industrial goal set out in Chapter 1. Following this, recommendations for future research are made. This research work will make a significant contribution to existing research on the ageing of oil spills. Chapter 6 discusses the criteria associated with ageing the release of kerosene in home heating oil spills by the development of the Power Regression Kerosene Model. This power model was based on the relationship between the normal alkane (nC_{14}) and the isoprenoid (iC_{16}), where the tetradecane (nC_{14}) : 2,6,10-trimethyl-tridecane (iC_{16}) ratio is plotted against the known number of days following a kerosene release. This industry led objective was answered in Chapter 6 by providing a model which will improve the capability of accurate allocation of responsibility to insurance companies following kerosene releases.

The primary aim of this thesis was to develop the model discussed in Chapter 6 and to establish an analytical protocol for chemical fingerprinting of spilled kerosene (domestic home heating oil). In order to meet this objective the research addressed a number of specific aims and objectives throughout the chapters. The six research questions addressed within this thesis were as follows.

7.1 Kerosene Weathering Experiment

To evaluate the preferential depletion of the n-alkanes: dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and tetradecane ($C_{14}H_{30}$) to the isoprenoids: iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) in the kerosene oil, a series of artificial weathering experiments were conducted and the findings are presented in Chapter 4. Within Chapter 4 is the evidence of the chemical profiling of kerosene by both gas chromatography coupled with flame ionisation detection, and gas chromatography coupled with mass spectrometry, which identified the individual n-alkanes and isoprenoids used in the power age estimation model. The mass spectra of the compounds tetradecane (nC_{14}) and 2,6,10-trimethyl-tridecane (iC_{16}) were compared to the NIST library. The weathering experiment evidence in Chapter 4 was further discussed in Chapter 6 where the findings concluded a preferential depletion of the n-alkane tetradecane (nC_{14}) over 2,6,10-trimethyl-tridecane (iC_{16}) when compared to evaporation levels. The major limitation of the kerosene weathering experiment relates to the matrix type which was assessed during the experiment. It would be advantageous to perform a number of laboratory bench studies looking at a range of different matrix types to help better inform the mechanism of preferential alteration of the n-alkane over the isoprenoids. This would also help to establish the soil matrix type boundaries for the use of the model.

7.2 Analytical Method for Extraction of Chromatographic Data

Within Chapter 5, it was necessary to develop an analytical method for the rapid extraction of isoprenoid and n-alkane content from chromatographic data. This was necessary due to the large volume of chromatographic data reviewed within the study (6,100 chromatograms). The purpose of the guide presented in Appendix 2 was to have a tool which could manage the volume of data that was required for the kerosene database (Appendix 3). The guide was developed to allow laboratory technicians in the future to accurately and consistently reproduce the data collection process used within this study. It is always important to establish tools which make the handling of large data sets easier to manipulate.

7.3 Development of Kerosene Data Base

The developed database of chromatograms and associated metadata was obtained from a review of over 6,100 samples received for analysis between 2009 and 2011 at Jones Environmental Forensics Limited Laboratory from a variety of environmental consultancy firms. These samples were submitted for analysis by environmental consultants working on domestic home heating oil releases in Ireland and the United Kingdom. Chapter 3 describes the methodology involved in the data gathering process, which involved a review of the Jones Environmental Forensics Ltd. final reports produced for each of the environmental consultants. Finally, once the database was developed each consultant was contacted and a variety of information including suspected release date and soil type was established. To improve this process of collecting the required data for the use of the model it would be advantageous to have an upfront discussion with the consultants. This approach would establish work plans where a variety of appropriate meta data and unstructured data could be discussed and included within the laboratories certified report.

7.4 Environmental Factors

Chapter 6 discusses all of the environmental factors which may influence the performance of the selected kerosene age estimation model. The primary two mechanisms driving the alteration of the ratio were evaporation and biodegradation. The second mechanism of alteration of the ratio was biodegradation and this was evident in the model represented by a curve. Within Chapter 6 the database of isoprenoid and n-alkane data of kerosene spill samples (soil) was evaluated. This evaluation informed the criteria and limitations associated with the use of the model. The environmental factors (weathering and biodegradation) affecting isoprenoids and n-alkane ratios in post-spill kerosene, which must be considered in order to accurately age a spill event were discussed in Chapter 6. The model in the first few days following release appeared to be affected by weathering (volatilisation) where the lighter n-alkanes were impacted over the first few days following release. This appeared to be exaggerated as the depth of the sample was closer to the surface.

7.5 Model Selection and Evaluation

The purpose of the research was to develop an equation equivalent to the Christensen and Larsen (1993), Kaplan, Galperin, Alimi et al., (1996b) equation used for middle distillate, based on forensic markers of isoprenoids and n-alkanes present in kerosene, which would be applicable to kerosene spills. The findings of the evaluation of the five models (Linear, exponential, power, logarithmic and polynomial) are presented in Chapter 5. Chapter 6 discusses each of the models trend lines associated with the plot of the ratio of tetradecane (nC_{14}) to 2,6,10-trimethyl-tridecane (iC_{16}) versus the number of days following release. A cross-validation, leave-one-out approach, described in Chapter 3, is undertaken and the findings for each model are presented in Chapter 5. Using descriptive statistics (MSE) each model was compared to the Christensen and Larsen (1993) and Hurst and Schmidt (2005) model. The eliminated sample numbers were reviewed in Chapter 6 to establish environmental factors to justify their removal from the final model. The power regression model was selected as the most appropriate model which best represented the mechanism driving the alteration of the ratio.

7.6 Power Regression Model Conditions of Use

It was important to consider the factors within the soil matrix upon which the kerosene spill ageing equation is reliant. Chapter 6 outlines these factors which include kerosene EPH concentration, sample collection minimum depth in meters, the presence in the sample analysis of the n-alkane (tetradecane ($nC_{14}H_{30}$)) and the isoprenoid (2,6,10-trimethyl-tridecane (iC_{16})), soil types for which the model can be used, and the range of %NMC.

7.7 Recommendations for Further Research

The potential for future research is great as there are a number of areas where the model could possibly be improved. In Chapter 6, Nagel (2012) an environmental consultant was quoted *"the cost of the additional analysis (pH, sulphide, temperature, microbial population and nutrients) is not really a factor as in the vast majority of cases as [the consultancy in question] do not need to get involved in the extent of the breakdown or degradation of kerosene within the soil therefore the extra analysis parameters would not be of benefit."* However, with the parameters mentioned above in mind, it is felt that it would be advantageous to review the influences of these environmental conditions on the model through a series of laboratory based trials.

For future research a laboratory experiment which could evaluate a variety of different soil types with varying chemical and physical properties and microbial populations would add value to the model and potentially refine the criteria associated with its use. With this additional physical, microbial and chemical knowledge, multi-variant statistics may be employed to add additional confidence to establish the abiotic and biotic factors influencing the model.

The analytical method for the rapid extraction of data from the chromatograms only applies to Agilent technologies gas chromatography equipment using Chemstation software. A review of the software such as Chromeleon used for data collection on other analytical instrumentation such as PerkinElmer and Thermo Scientific systems and their associated software packages would be advantageous as not all laboratories use Agilent Technology systems.

It would be advantageous to conduct an Inter-Laboratory Comparison which would perform an evaluation of the test methodology employed by different laboratories in accordance with predetermined conditions. These conditions may include soil type, variety of nutrient and kerosene concentrations at different levels of weathering alterations and would determine the reproducibility of the model across different laboratories.

8.0 Appendix 1 Extractable Petroleum Hydrocarbons Analytical Method

The analytical extraction and instrument conditions described in this appendix are taken from Jones Environmental Forensics Ltd's United Kingdom Accreditation Service (UKAS) accredited methods for Extractable Petroleum Hydrocarbon and Whole oil methods. The methodologies within this Appendix are taken from:

- TM001 Whole Oil Analysis v8,
- TM005 EPH (inc CWG) by GC-FID v11, and
- PM008S Extraction by End over End Shaker V9.

8.1 Extractable Petroleum Hydrocarbons Analytical: Method "End-Over-End" Shaker Extraction

This method describes the procedure for the extraction of an 'as received' soil sample for analysis for EPH in the carbon range octane (nC_8) to tetracontane (nC_{40}) using "end-over-end" shaker extraction process.

8.1.1 Reagents

- n-Hexane, 95% min. purity e.g. J.T. Baker
- Acetone, 99.8% min. purity e.g. HiPerSolv
- Dichloromethane (DCM), 99.9% e.g. Fluka
- Copper granules, high purity e.g. Sci-lab
- Sodium Sulphate, 90% min purity, e.g. J.T.Bake

8.1.2 Equipment

- Stuart STR4 "end-over-end" rotator shaker
- Top-pan balance capable of weighing to 0.1g
- Dispenser capable of dispensing 10ml and 20ml of solvent
- 1000ml Class B measuring cylinder
- 500µl and 100µl precision syringe
- 60ml, 40ml, 7.5ml and 2ml vials
- Crimp caps for 2ml vials
- Crimping tool
- Autopipette capable of pipetting 1000µl

- Disposable pipette tips
- Spatula
- Pen capable of writing on glass
- Tray for 2ml, 40ml and 60ml vials

8.1.3 Reagent Preparation – Hexane : Acetone (1:1)

One litre (1L) of each of the solvents is measured into a clean one litre measuring cylinder and transferred to a dispenser bottle. The dispenser bottle must be clearly labelled 'Hexane : Acetone (1:1)' with the preparation date, initials, and an expiry date of one month from the date prepared.

8.1.4 Preparation of Soil Samples

The sample identification number is labelled on to a clean 60ml vial. To this vial 5g $\pm$ 0.2g of soil sample is weighed using a top pan balance. The weight is recorded on an extraction worksheet. The sample is then spiked with 100µl of Squalene (5,000 mg/l) using a precision syringe. Approximately 2g of sodium sulphate is added to the same vial to dry the soil. 20ml of Hexane: Acetone (1:1) is added to the vial using a dispenser and the vial is shaken well. The vial is securely fitted into place on the Stuart STR4 "end-over-end" rotator shaker (Figure 8.1) so that it is balanced on all four sides. The "end-over-end" rotator shaker is set to '3' (a gentle turning motion) and the samples is rotated end over end for 45 minutes.

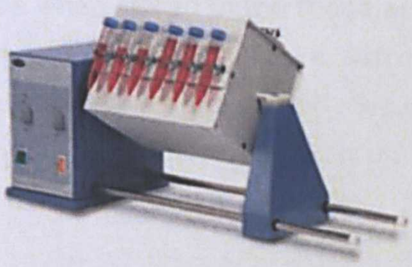


Figure 8.1 Stuart STR4 Drive Unit Rotator "End-Over-End"

After 45 minutes the sample(s) are taken off the Stuart STR4 "end-over-end" rotator shaker and are filled with cold tap water to the neck of the vial, the lids are securely fitted and all vials shaken well. The addition of water allows the acetone portion of the solvent to disperse into the water layer, leaving a visible extract layer of hexane at the top of the vial. This is a critical step of the method and not performing this will adversely affect results. The vial is then left to settle for at least 5-10 minutes. 1ml of the extract hexane layer is carefully transferred from the vial into a labelled 2ml vial using an autopipette with a new disposable tip and then crimped closed. The extract is inspected visually and if an extract is found to contain suspended matter, or has formed an emulsion, then the extract layer is transferred into a 7.5ml labelled vial and small volumes are transferred into a 2ml labelled vial, taking care to avoid the suspended matter. It is important to obtain a clear extract. 1ml of the extract is then transferred from the 7.5ml vial into the 2ml labelled vial for analysis. The sample(s) are then ready for analysis.

8.2 Extractable Petroleum Hydrocarbons Analytical Instrument: Gas Chromatography Procedure

8.2.1 Reference Material

- Commercially available Kerosene from two separate sources – one is used to prepare working EPH external calibration standards and linear check standards, the other to prepare AQC standards and Independent standards. The shelf life is 4 years from receipt when stored in the fridge at 5 ± 4°C.
- 500µg/ml (each component) total recoverable petroleum hydrocarbon standard (TRPH), octane (nC_8) to tetracontane (nC_{40}) (Florida Mix). This is used as a retention time standard (RTS). The shelf life is stated on the certificate of analysis when stored in the fridge at 5 ± 4°C
- 98% purity squalene. This is used as a surrogate spike added at the extraction stage described in paragraph 8.1.4 of the preparation of samples for analysis. The shelf life is stated on the Certificate of Analysis when stored in the fridge at 5 ± 4°C.

8.2.2 Reagents

- Helium gas (carrier), 99.995% purity min
- Air / service air, compressed gas, -40°C dew point
- Hydrogen and Nitrogen, 99.99% purity min
- Dichloromethane, 99.5% min

- n-Hexane, 95% min, or equivalent

8.2.3 Equipment

Gas Chromatographic Agilent 6890 or 7890 GCFID.

- Varian fused silica mineral oil capillary column 15m x 0.32mm ID x 0.1µm film
- 2.0 ml clear, write-on crimp top vials
- 20ml, 40ml clear screw top vials
- 10ml, 20ml and 100ml class B volumetric flasks
- 100µl, 500µl and 1000µl precision syringes
- Analytical balance capable of weighing to 0.0001 g

8.2.4 Preparation of Stock Kerosene Standard – 10,000mg/l

0.5g of kerosene is accurately weighed into a clean 50ml volumetric flask. The volumetric flask is made up to the 50ml mark with n-Hexane, mixed thoroughly, and the contents transferred to a screw-capped reagent vial. This process is repeated using one lot for analytical quality control (AQC), and one for calibration stock. These stock standards are then used to prepare the calibration and AQC standards. Shelf life is six weeks when refrigerated at 5 ± 4°C.

8.2.5 Preparation of Surrogate Standard for Extraction Step – 5,000 mg/l Squalene

To approximately 50mls of n-hexane add 500µl of squalene into a clean 100ml volumetric flask. Make up to the 100ml mark with n-hexane, mix thoroughly and transfer to screw-capped reagent vials. Transfer to 2ml labelled vials for the extraction step as necessary. Shelf life is 1 month when refrigerated at 5 ± 4°C.

8.2.6 Preparation of Retention Time Standard: 1,275 mg/l

Add 1.5ml of the Total Recoverable Petroleum standard mix to a clean 10ml volumetric flask. Make up to the 10ml mark with n-hexane, cap securely and mix thoroughly. Transfer to screw-capped reagent vials and transfer to 2ml labelled vials for analysis as necessary. Shelf life is 3 months when refrigerated at 5 ± 4°C.

8.2.7 Preparation of Kerosene Calibration Standards

Prepare 20ml of each of the following EPH kerosene standards in the total concentration range 50mg/l to 5,000mg/l as follows (Table 8.1). Shelf life is six weeks when refrigerated at 5 ± 4°C.

Table 8.1 Preparation of EPH Kerosene Calibration Standards

Total Concentration of EPH Calibration Standard (mg/l)	Concentration of Squalene (mg/l)	µl of 10,000 mg/l stock Kerosene EPH	µl of 5,000 mg/l stock Squalene
50	5	100	20
100	10	200	40
500	25	1,000	100
1,000	50	2,000	200
2,500	75	5,000	300
5,000	100	10,000	400
AQC (2,000)	50	4,000	200

Using the appropriate precision syringe, add the required volume of stock kerosene EPH standard to a 20ml volumetric flask containing approximately 10mls of n-hexane and make up to the measure mark with n-hexane solvent. The calibration and AQC standards are transferred to screw cap vials and transferred to 2ml labelled vials for analysis as necessary.

8.2.8 Calculation of Results

The information on the extraction worksheet is taken into account when calculating the result of the samples e.g. dilution factor, amount of sample. A sample multiplier is calculated for each sample as follows (Equation 12):

$$Multiplier = \frac{\text{Volume of Extract (ml)}}{\text{Sample Weight (g)}} \text{ x dilution factor}$$

Equation 12 Multiplier Equation

The following calculations are performed using the chromatographic software. The calculation for each of the standards is undertaken by obtaining the total area under the chromatogram (with the appropriate time window), after the baseline has been drawn, for each level of calibration, calculate the response factor as follows (Equation 13):

$$Response\ Factor = \frac{\text{Total Area under Chromatogram for Calibration Level}}{\text{Concentration of Calibration Level (mg/l)}}$$

Equation 13 Response Factor

Note: The chromatography software will construct an external calibration curve of concentration versus area for the analytes in question (Equation 14).

$$Analyte, mg/kg = \frac{C(x) \times V(e) \times DF}{W(s)}$$

Where:

C(x) - concentration of analyte in question, generated by the chromatography software, mg/l

V(e) - volume of extract, ml or sample weight, g

DF - dilution factor, if further dilution required

W(s) - original weight/volume of sample extracted, g or ml

Equation 14 Analyte Concentration Equation

8.2.9 Quality Control Checks

A calibration check standard was analysed at the start and end of every run of samples in order to verify the linearity of the six point external calibration. The selection of different check standards at different levels was done to assess the whole of the calibration range for linearity. These check standards were prepared in the same way as the initial external calibration. The chromatographic software permits the construction of external calibration curves for each fraction of the sample extract such as for kerosene and for any surrogates. The calibration curve slopes all fall within the set criteria, with a correlation coefficient of 0.995 or better. A surrogate spike was added at the extraction stage to all samples including the extracted AQC and a blank. This was used as a quality check to ensure successful extraction process. The typical acceptance limits are 70 - 130% recovery.

8.2.10 System Suitability Check

Good chromatography is described as having peaks that are gaussian in nature. Background subtraction, if required, is used to eliminate column bleed, electrical spikes or instrument background noise. A peak asymmetry calculation was used as part of the chromatographic software to calculate the peak tailing and/or fronting factor. For ideal peaks the asymmetry (A) = 1. A Retention Time Standard was analysed with every run of samples and the asymmetry of peak pentadecane (nC_{15}) was monitored. Any failure indicates that the chromatography was unacceptable, maintenance was performed and all samples in that run were repeated.

8.3 Whole Oil Analytical Method

8.3.1 Scope

This procedure describes the bulk characteristics, distillation range and the subsequent banding of pure products and aliphatic and aromatic fractions by Gas Chromatography (GC) with flame ionisation detection (FID). The carbon number range detected by correct application of this method is pentane (nC_5) to triacontane plus (nC_{35+}). For the aliphatic/aromatic bands the carbon range is hexane (nC_6) to triacontane plus (nC_{35+}).

8.3.2 Principle

A product, aliphatic/aromatic fraction or solvent extract is directly injected onto a gas chromatograph and is analysed by temperature programmed capillary chromatography and flame ionisation detection (FID). The chromatogram obtained serves as a "fingerprint" of the sample components and allows the determination of the bulk characteristic/possible distillation range of the sample. In order to assess the distillation/boiling range of the sample, a sample of crude oil is analysed daily and the retention times of the n-alkanes therein are compared to those within the sample. The pattern produced in the sample chromatogram is best matched to a series of 'in-house' reference materials, which have been analysed previously under the same GC-FID conditions

8.3.3 Reference Materials

All standards are stored at a temperature of 5°C +/- 4°C with expiry dates of 4 years from receipt

- Typical sample of Forties Blend Crude Oil
- Typical sample of Ultra Low Sulphur Diesel
- Typical Unleaded Gasoline
- Typical sample of Kerosene
- Typical sample of Lubricating Oil
- Typical sample of White Spirit
- Typical sample of Creosote
- Typical sample of Pink Paraffin
- Typical sample of Jeyes Fluid
- Typical sample of Hydraulic Brake Fluid
- Typical sample of Jet A-1 Aviation Fuel
- Typical sample of Transformer Oil
- Typical sample of Antifreeze (containing Ethylene Glycol)
- Typical sample of 35 second Heating Oil (Red Diesel)
- Typical sample of Rapeseed Oil
- Typical sample of Linseed Oil

8.3.4 Reagents

- Helium gas (carrier) – 99.995% purity min.
- Air/service air – compressed gas - -40°C dew point
- Hydrogen – 99.995% purity min.
- Dichloromethane – HPLC grade
- Forties crude oil
- Sodium sulphate

8.3.5 Equipment

Gas chromatographic system equipped with flame ionisation detector, and autosampler capable of operating according to the conditions given in paragraph 8.3.12 - GC FID Agilent 6850 Operating Conditions.

8.3.6 Interferences

Electrical spikes can occur resulting in the appearance of 'spikes' rather than peaks. These spikes may also present as contamination resulting from poor handling of equipment. To avoid cross contamination it is essential that injection needle and the septum are not touched. If the problem is persistent it may be necessary to carry out maintenance. Contamination by carry over can occur whenever high-level and low-level samples are sequentially analysed. As part of the sampling sequence of the GC-FID, the syringe must be rinsed out between samples with solvent (Dichloromethane). Whenever an unusually concentrated sample is encountered, the next analysis should be that of a solvent blank wherever possible i.e. Dichloromethane, to ensure that no cross-contamination occurs. Where the sample has been analysed as part of a batch, the sample itself and any of the following samples which may contain carryover must be repeated.

8.3.7 Sample Preparation - Preparation of product samples

Product samples and aliphatic/aromatic fractions are transferred to a 2ml crimp top vial and capped securely prior to GC analysis. A viscous sample may require a small dilution in a suitable organic solvent such as dichloromethane prior to GC analysis. This dilution depends on how viscous the sample is and is purely to allow the sample to be drawn up the autosampler syringe. Sodium sulphate is added to desiccate a sample if it has been in contact with water e.g. when a thin layer of oil is taken off a water sample.

8.3.8 Quality Control Checks

A dichloromethane wash is run at the beginning of every sequence to ensure the instrument is clean. A Profile sample is run every 10 samples and subtracted from the baseline to eliminate column bleed, electrical spikes, instrument background noise, and to ensure the cleanliness of the GC. A Crude Oil is run at the start of a run, then every ten samples, and again at the end of the run, to ensure no shift in retention times and to monitor the chromatography. After the crude oil has been run, the vial is recapped as soon as possible to prevent any front end losses. The following samples in the sequence are checked and re-run if they are found to be contaminated by carry-over.

8.3.9 System Suitability Check

The peak asymmetry calculation is used as part of the Chemstation software to calculate the peak tailing and/or fronting factor. For ideal peaks the asymmetry (A) = 1. A crude oil is run every ten samples and the asymmetry of peak octadecane (nC_{18}) for each one is plotted on a system suitability chart (SSC). A failure indicates that the chromatography is unacceptable and maintenance is carried out. All samples are repeated. In order to assess the condition and suitability of the GC a sample of Forties crude oil is analysed every ten samples. The fingerprint obtained should have greater than 1.5 resolution (baseline resolution) between the closely eluting peaks of heptadecane (nC_{17}) / Pristane and octadecane (nC_{18}) / Phytane. It is then plotted on a SSC chart. The lower critical limit on the SSC chart is set at 0.5. The upper critical limit is set at 1.5. One point below/above these lines is acceptable but must be investigated as to the cause. Two consecutive points below/above these lines constitute a failure and all samples relating to the failure must be re-run. Good chromatography is described as having peaks that are gaussian in nature. If poor chromatography is noted this may indicate that the deactivated glass liner may need to be cleaned and/or replaced or the whole injection port may require cleaning. It may also be necessary to perform a 'column chop' which involves the removal of 6-12 inches of capillary column adjacent to the injector. If poor chromatography persists after this, it might be necessary to perform a complete column change or injector/detector clean. Instructions for column change and injector/detector cleans are outlined in the Agilent 6850 Operator's manual. These procedures are an integral part of routine maintenance of the GC-FID system.

If any GC maintenance is carried out it will be necessary to analyse the Forties crude before running any samples. Any maintenance performed, either routine or non-routine, should be recorded in the Instrument Maintenance Log in the instrument file located next to the instrument.

8.3.10 Calculation of Results

It should be noted that this is a subjective assessment because the chromatograms of the 'in house' reference materials can only be classed as "typical" of the respective product, such that two samples of the same product from different sources may display different fingerprint characteristics. The sample chromatograms obtained using this method are qualitative only and are subsequently compared to the 'in house' reference material chromatograms in order to allow an assessment of the characteristics of the sample to be made. Example chromatograms of typical products are kept in the 'Reference Standards' file. From the sample chromatogram and peak data obtained it is possible to calculate several diagnostic ratios such as heptadecane (nC_{17}) : Pristane, octadecane (nC_{18}) : Phytane and Pristane: Phytane. In cases where the product shows similar characteristics to diesel, the heptadecane (nC_{17}) : Pristane ratio can be further used to calculate the age of the spill in samples.

- Pristane /Phytane Ratio - Ratio = Peak height of Pristane / Peak height of Phytane
- Heptadecane (nC_{17}) : Pristane Ratio - Ratio = Peak height of Heptadecane (nC_{17}) / Peak height of Pristane
- Octadecane (nC_{18}) : Phytane Ratio - Ratio = Peak height of octadecane (nC_{18}) / Peak height of Phytane
- Heptadecane (nC_{17}) : Pristane Ratio Age Formula (for diesel) T (Time, +/- 2years) = -8.4 x (Heptadecane (nC_{17}) / Pristane) + 19.8
- Heptadecane (nC_{17}) / Pristane and octadecane (nC_{18}) / Phytane Resolution - $R = \frac{2(T_{R(b)} - T_{R(a)})}{W_{B(B)} + W_{B(a)}}$ where: TR(x) is the retention time of peak (x) and WB(x) is the base width for peak (x) in min

Source: Agilent Technologies Document; 'Evaluating System Suitability CE, GC, LC and A/D Chemstation. Revisions: A.03.0x- A.08.0x'

- Peak Asymmetry Calculation (EP Standard) – $A = \frac{RW_{5\%} + LW_{5\%}}{2 * LW_{5\%}}$ where $RW_{5\%}$ is the right width of baselined peak at 5% height and $LW_{5\%}$ is the left width of baselined peak at 5% height. Note: This calculation is performed as part of the Chemstation software
- Calculation of Results for Aliphatic / Aromatic Fractionation - The total area of the hexane (nC_6) to tricontane (nC_{35}) portion of the chromatogram is obtained for each fraction and the aliphatic/aromatic split is determined as follows:

$$\% \, Aliphatic = \frac{Area \; of \; Aliphatic \; (nC_6 \; to \; nC_{35})}{Total \; Area \; of \; Aliphatic + Total \; Area \; of \; Aromatics)}$$

$$\% \, Aromatics = \frac{Area \; of \; Aromatics \; (C_6 \; to \; C_{35})}{Total \; Area \; of \; Aliphatic + Total \; Area \; of \; Aromatics)}$$

The data obtained from the carbon bands is recalculated using the following:

- Aliphatic C6-C8 = % in band x % aliphatics in oil
- Aromatics EC6-EC8 = % in band x % aromatics in oil

8.3.11 Reports

An interpretation of the nature of the sample material, general description, the diagnostic ratios and calculated age of the sample where possible, are reported on the 'Whole Oil Template'. Note: Opinions and interpretations are outside the scope of UKAS accreditation

8.3.12 GC FID Agilent 6850 Operating Conditions

8.3.12.1 Column

- Material - Fused Silica Capillary Column (DB-1)
- Dimensions - 50m x 0.25mm (I.D) x 0.25µm (Film Thickness)
- Gases - Helium: constant flow of 1.0 ml/min

8.3.12.2 Detector

- Type – FID
- Temperature – 350 °C
- Signal - 0

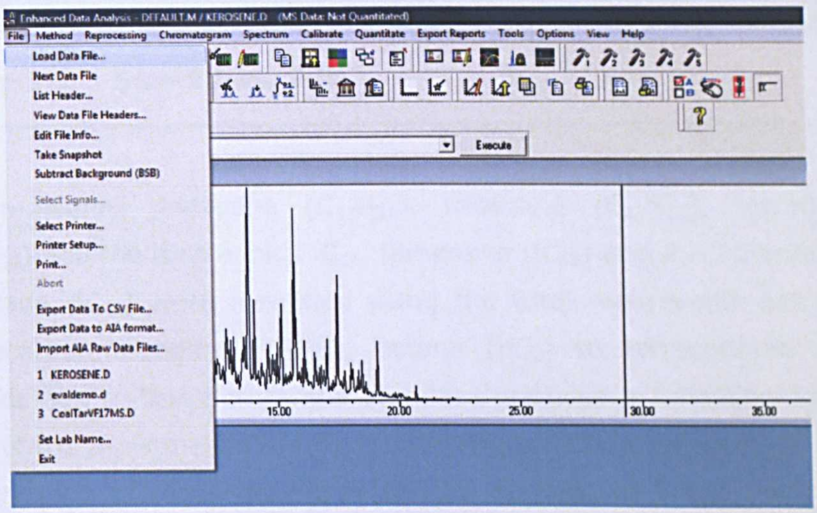
8.3.12.3 Oven

Initial temperature of 40°C for 5 minutes, ramp at 8°C/min to 200°C and hold for 1 minute, finally ramp at 10°C / min to 340°C and hold for 20 minutes.

8.3.12.4 Injection

Split 150:1, Volume Injection 1.0µl, Inlet temperature 320°C, Pressure 25.0 psi, gas Helium.

9.0 Appendix 2 Integration Procedure for Gathering Data on Fingerprint Chromatograms of Kerosene

Step No.	Procedure
Step 1	Startup and open the Enhanced Data Analysis Chemstation software. From the menu bar at the top select "File" and from the dropdown list provided select "Load data file" (Figure 9.1).  **Figure 9.1 File and Load Data File**
Step 2	Step 1, opens a window "Select Data File" where the chromatogram data files can be selected for review. A snapshot fingerprint along with the operator's name, vial position number and sample name is provided within this window. In the left column of the "Select Data File" window a list of all the data files are presented. Click on the data file of interest and click the "OK" button to load and open this file into the software for integration (Figure 9.2).

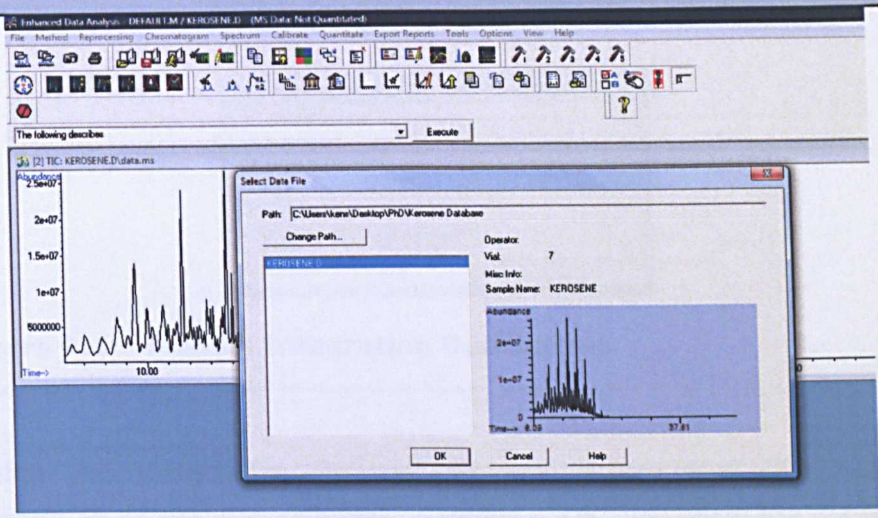
<table>
<tr><th>Step No.</th><th>Procedure</th></tr>
<tr><td></td><td>


Figure 9.2 Select Data File
</td></tr>
<tr><td>Step 3</td><td>
The n-alkanes dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$), tetradecane ($C_{14}H_{30}$) and the isoprenoids iC_{14}, farnesane (iC_{15}) and 2,6,10-trimethyl-tridecane (iC_{16}) were identified using the total recoverable petroleum hydrocarbon standard (TRPH), octane (nC_8) to tetracontane (nC_{40}) (Florida Mix). This florida mix standard provides a retention time for each of the n-alkanes in the kerosene sample to be identified. A further confirmation of these n-alkanes can be carried out when using mass spectrometry detection techniques by using the national institute of standards and technology (NIST) library. From the menu bar select "Tools" and from the dropdown list provided select "Options" (Figure 9.3). This opens a window called "Select DA Options" (Figure 9.4).

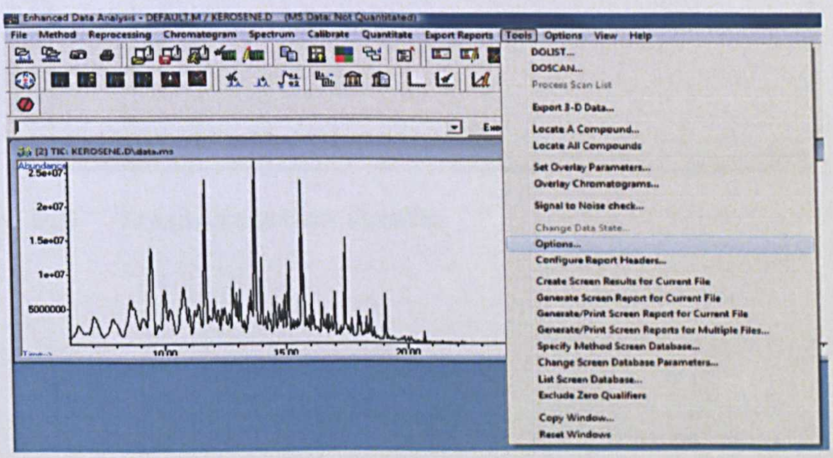


Figure 9.3 Manual Integration
</td></tr>
</table>

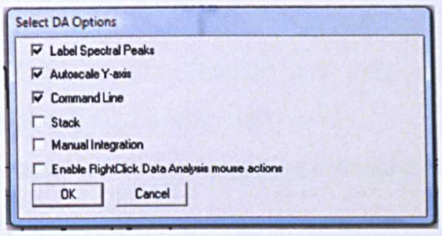
<table>
<tr><th>Step No.</th><th>Procedure</th></tr>
<tr><td></td><td>

Figure 9.4 Manual Integration Deselected</td></tr>
<tr><td>Step 4</td><td>Within the "Select DA Options" window ensure that the Manual Integration option is deselected. Selecting "OK" will allow the software to perform a search against the NIST library. Move the cursor over the peak with the retention time for dodecane and double click the right button on the mouse. This will open a second window which contains the fragmentation profile for the peak at the retention time selected (Figure 9.5).

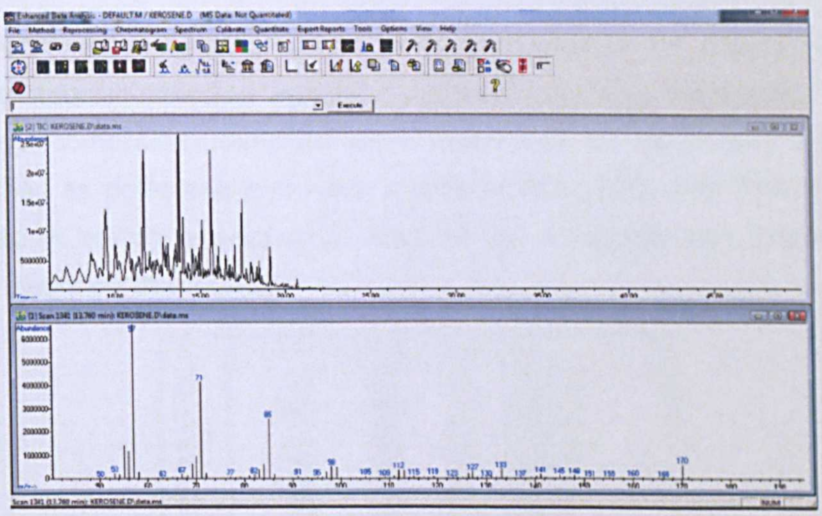



Figure 9.5 Fragmentation Profile</td></tr>
</table>

Step No.	Procedure

Step 5

By moving the mouse down over the fragmentation profile and double clicking the right button on the mouse this will perform a search into the NIST library (Figure 9.6) by the software.

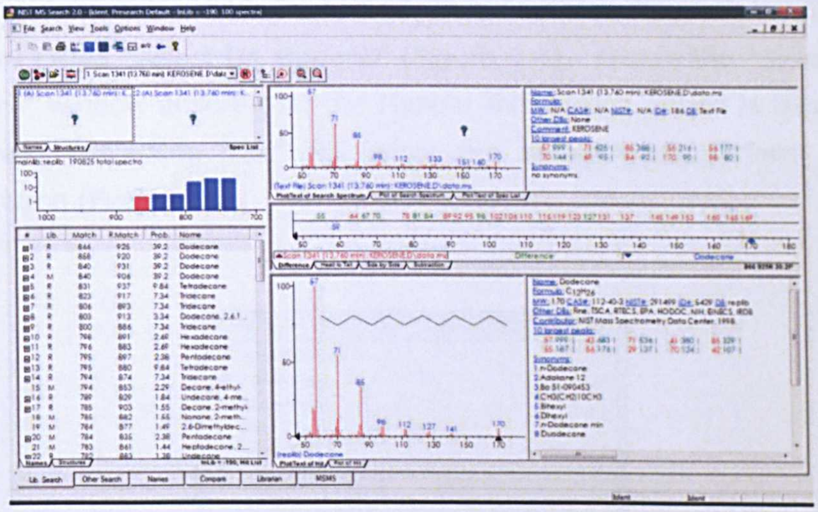
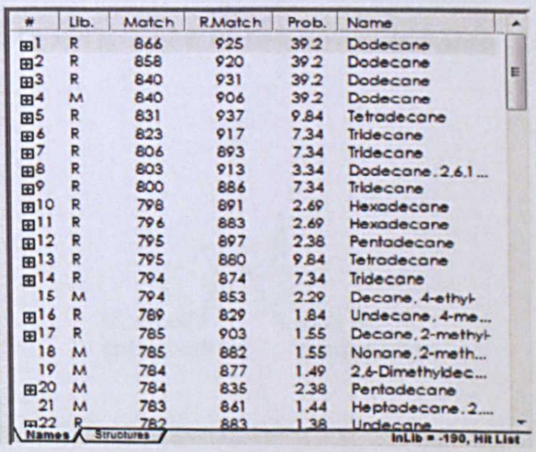


Figure 9.6 NIST Library Search Results

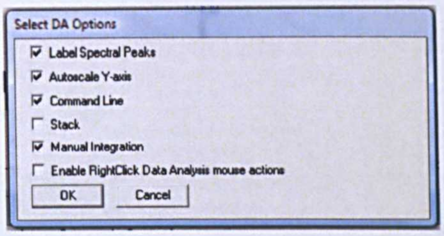
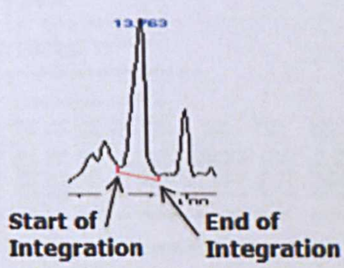
Step 6

The NIST MS search provides a fit and reverse fit match of the fragmentogram selected against fragmentogram's in the library. The first four compounds identified within the search hit list (Figure 9.7) are identified as dodecane and have a greater than 80% and 90% fit and reverse fit match respectively. Each of the n-alkanes and isoprenoids were identified in this way.



#	Lib.	Match	R.Match	Prob.	Name
1	R	866	925	39.2	Dodecane
2	R	858	920	39.2	Dodecane
3	R	840	931	39.2	Dodecane
4	M	840	906	39.2	Dodecane
5	R	831	937	9.84	Tetradecane
6	R	823	917	7.34	Tridecane
7	R	806	893	7.34	Tridecane
8	R	803	913	3.34	Dodecane, 2,6,1...
9	R	800	886	7.34	Tridecane
10	R	798	891	2.69	Hexadecane
11	R	796	883	2.69	Hexadecane
12	R	795	897	2.38	Pentadecane
13	R	795	880	9.84	Tetradecane
14	R	794	874	7.34	Tridecane
15	M	794	853	2.29	Decane, 4-ethyl-
16	R	789	829	1.84	Undecane, 4-me...
17	R	785	903	1.55	Decane, 2-methyl-
18	M	785	882	1.55	Nonane, 2-meth...
19	M	784	877	1.49	2,6-Dimethyldec...
20	M	784	835	2.38	Pentadecane
21	M	783	861	1.44	Heptadecane, 2....
22	R	782	883	1.38	Undecane

Figure 9.7 NIST Hit List

Step No.	Procedure
Step 7	Each of the n-alkanes and isoprenoids are identified in this way. To determine the peak height of each of the compounds of interest the following steps are taken. **From the menu bar select "Tools" and from the dropdown list provided select "Options"** (Figure 9.3). This opens a **window called "Select DA Options"** (Figure 9.4). **Within the "Select DA Options" window** ensure that the Manual Integration option is this time selected. **Selecting "OK" will allow the software to perform peak** integration (Figure 9.8).  **Figure 9.8 Manual Integration Selected**
Step 8	It is possible with the software to expand in on the area of interest within the chromatogram. This allows for easy integration of the n-alkanes and isoprenoids. Using the left button on the mouse select the area within the chromatogram of interest and on the release of the mouse button the area is expanded into the window. To integrate a peak, select the start of the peak by holding down the left button on the mouse and extending a line to the end of the peak (Figure 9.9).  **Figure 9.9 Peak Integration**

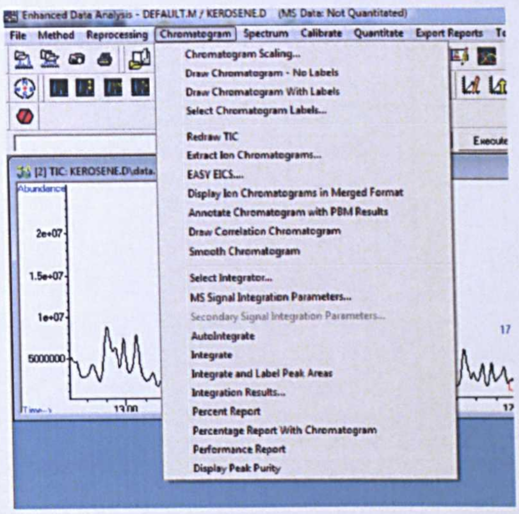
<table>
<tr><th>Step No.</th><th>Procedure</th></tr>
<tr><td>Step 9</td><td>Once all of the peaks are integrated from the menu bar select "Chromatogram" and from the dropdown list select "Percent Report" (Figure 9.10).


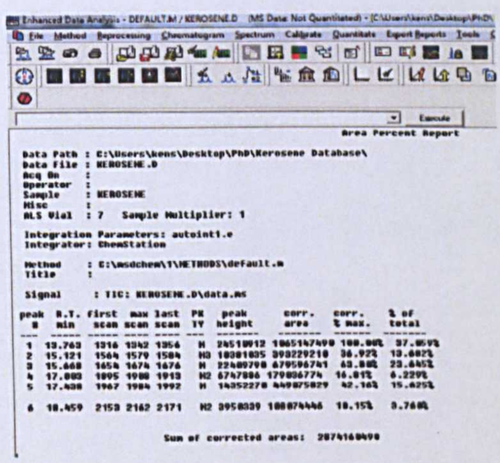
Figure 9.10 Chromatogram – Percent Report</td></tr>
<tr><td>Step 10</td><td>This area percent report includes the data file name, and the peak height information for the six peaks which were selected during the integration (Figure 9.11).


Figure 9.11 Area Percent Report</td></tr>
</table>

<table>
<tr><th>Step No.</th><th>Procedure</th></tr>
<tr><td>Step 11</td><td>
To export this data into an excel format for further manipulation, from the menu bar select "File" and from the dropdown list select "Export Data To CSV File" (Figure 9.12). This opens a window named "Select Export Mode" (Figure 9.13). Within the "Select Export Mode" window ensure that the "Current File – Select Destination" option is selected.

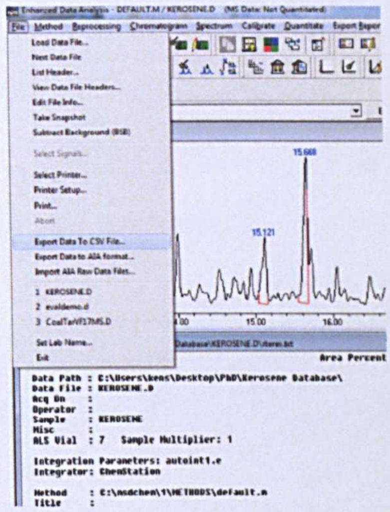


Figure 9.12 Export Data To Comma-separated Value (CSV) File

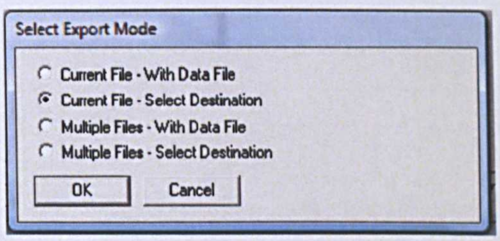
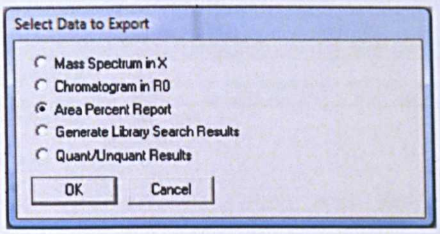
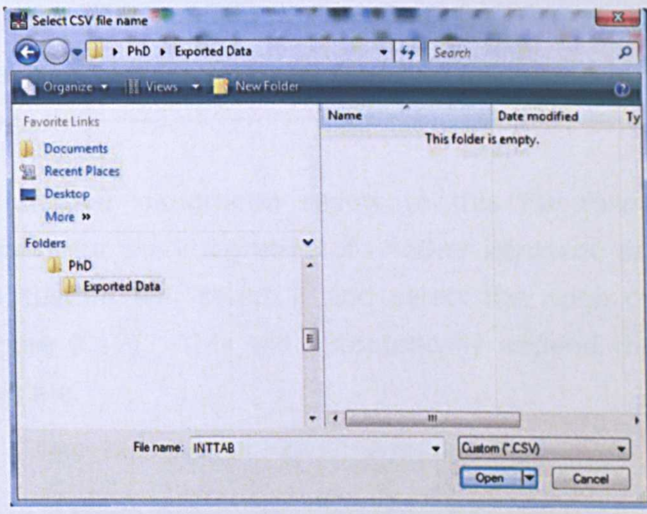


Figure 9.13 Select Export Mode
</td></tr>
</table>

Step No.	Procedure
Step 12	By selecting the "OK" button in this window it opens another window called "Select Data to Export" (Figure 9.14). Ensure that the "Area Percent Report" option is selected and select "OK" which will open a new window "Select CSV file name" (Figure 9.15).  **Figure 9.14 Select Data to Export**  **Figure 9.15 Select CSV File Name**

<table>
<tr><th>Step No.</th><th>Procedure</th></tr>
<tr><td>Step 13</td><td>

This option creates a custom (*.CSV) file named **"INTTAB"** where the data is exported into (Figure 9.16).

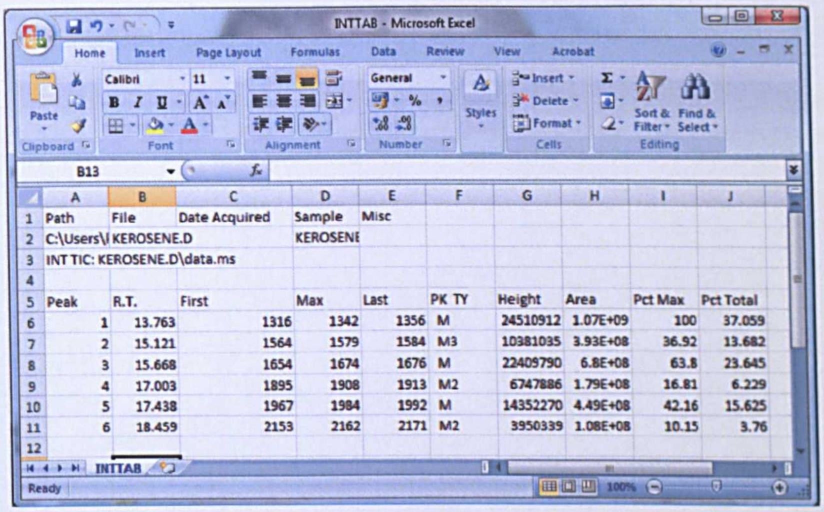


Figure 9.16 INTTAB – Microsoft Excel

</td></tr>
<tr><td>Step 14</td><td>

To append another integration report to this file follow the steps described above for the integration of another kerosene data file. Find the INTTAB custom file, select it and select the open option in this window (Figure 9.17). This will automatically append the integration data into this file.

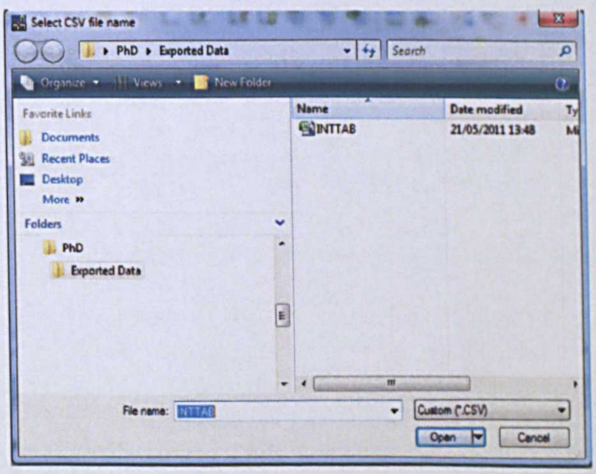


Figure 9.17 Select CSV File Name – Appended

</td></tr>
</table>

10.0 Appendix 3 Kerosene Database

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
1	Consultant 1	1.8-2.0	572	260522	52639	190558	44826	118958	29245	4.95	4.25	4.07	Unknown	23/09/2009
2	Consultant 2	-	41294	14361818	3106950	12283093	2569718	8169180	1874428	4.62	4.78	4.36	-	-
3	Consultant 2	-	3732	1555715	309805	1356104	232974	815747	156375	5.02	5.82	5.22	-	-
4	Consultant 2	-	24836	10355940	2354621	10136901	1997055	6876648	1457463	4.40	5.08	4.72	-	-
5	Consultant 2	-	35832	14634977	3279113	13316599	2670767	8879162	1892018	4.46	4.99	4.69	-	-
6	Consultant 2	-	19005	7909598	2073082	9321502	1961699	6703448	1433370	3.82	4.75	4.68	-	-
7	Consultant 2	-	20611	8597564	1991204	8319054	1575798	5389414	1127350	4.32	5.28	4.78	-	-
8	Consultant 2	-	3241	1227135	277212	998790	189544	532719	108292	4.43	5.27	4.92	-	-
9	Consultant 2	-	1745	806455	120927	607463	84217	297458	48384	6.67	7.21	6.15	-	-
10	Consultant 2	-	2377	889635	263694	877445	200209	540551	119532	3.37	4.38	4.52	-	-
11	Consultant 2	-	323	139200	19251	94934	13774	49026	9322	7.23	6.89	5.26	-	-
12	Consultant 1	0.4	164	54659	15318	38408	8294	15925	4404	3.57	4.63	3.62	Unknown	24/09/2009
13	Consultant 1	0.4	88	38822	13038	30125	7201	13748	4006	2.98	4.18	3.43	Unknown	24/09/2009
14	Consultant 1	0.7	443	137740	40997	90731	20617	37913	11143	3.36	4.40	3.40	Unknown	24/09/2009
15	Consultant 1	0.6	422	152021	59384	123483	41987	59669	24755	2.56	2.94	2.41	Unknown	24/09/2009
16	Consultant 1	0.8	3071	1055316	262606	661871	132191	261077	66432	4.02	5.01	3.93	Unknown	24/09/2009
17	Consultant 3	1	341	168020	46775	137289	32718	76864	21906	3.59	4.20	3.51	Unknown	09/09/2009
18	Consultant 4	0.75	14928	6838807	1298382	6271179	1149136	4816266	748208	5.27	5.46	6.44	28/09/2009	29/09/2009
19	Consultant 4	0	7760	2795397	790727	2552960	694957	1733481	459781	3.54	3.67	3.77	28/09/2009	29/09/2009
20	Consultant 4	0.5	4197	2260371	582374	1996258	505923	1334972	434046	3.88	3.95	3.08	Unknown	30/09/2009
21	Consultant 4	1.25	150	58524	12580	44958	12516	25174	7631	4.65	3.59	3.30	Unknown	30/09/2009
22	Consultant 2	-	8152	3156346	573535	2286541	604484	1303805	372744	5.50	3.78	3.50	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12} : iC_{14}$	$nC_{13} : iC_{15}$	$nC_{14} : iC_{16}$	Release Date	Sampling Date
23	Consultant 2	-	834	322961	70008	260731	54377	166933	35295	4.61	4.79	4.73	-	-
24	Consultant 2	-	840	258932	51886	184707	43932	118549	26846	4.99	4.20	4.42	-	-
25	Consultant 1	-	770	377273	74785	279665	52564	143955	31488	5.04	5.32	4.57	Unknown	26/09/2009
26	Consultant 1	-	886	385773	67601	256029	52088	126395	27163	5.71	4.92	4.65	Unknown	26/09/2009
27	Consultant 4	0.2	2619	1096207	206686	875557	141167	450360	78756	5.30	6.20	5.72	Validation Sample	-
28	Consultant 1	-	156	31777	14196	34817	10121	21152	7080	2.24	3.44	2.99	01/04/2009	30/09/2009
29	Consultant 1	-	287	62478	22264	53586	14579	31038	9877	2.81	3.68	3.14	01/04/2009	30/09/2009
30	Consultant 1	-	300	114914	50214	157538	40898	84192	24128	2.29	3.85	3.49	01/04/2009	30/09/2009
31	Consultant 1	0.5-0.9	453	68834	36994	66256	40359	44046	22659	1.86	1.64	1.94	Validation Sample	-
32	Consultant 1	0.5-0.9	2946	429165	127456	515266	148760	437604	110101	3.37	3.46	3.97	Validation Sample	-
33	Consultant 1	0.9	372	118178	40113	112377	38334	83807	24980	2.95	2.93	3.35	Validation Sample	-
34	Consultant 4	0.2	5874	2884998	671776	2381734	550129	1530836	350801	4.29	4.33	4.36	20/09/2009	29/09/2009
35	Consultant 4	0.4	3354	1660379	417797	1417352	329687	852616	203080	3.97	4.30	4.20	20/09/2009	29/09/2009
36	Consultant 4	0.4	9324	3944982	950147	3326830	754157	1931313	438740	4.15	4.41	4.40	20/09/2009	29/09/2009
37	Consultant 4	0.2	3501	1668691	375340	1449770	311653	860189	174324	4.45	4.65	4.93	19/09/2009	25/09/2009
38	Consultant 4	0.6	6216	2837624	515058	2098187	424188	1143024	251401	5.51	4.95	4.55	19/09/2009	25/09/2009
39	Consultant 4	0.4	4644	2513319	494240	1834269	414189	1029361	228766	5.09	4.43	4.50	19/09/2009	25/09/2009
40	Consultant 1	2.6-2.7	135	54236	21274	49536	18554	32245	16822	2.55	2.67	1.92	Unknown	05/10/2009
41	Consultant 1	2-2.2	4024	2373220	424441	1621893	338626	936215	274757	5.59	4.79	3.41	Unknown	05/10/2009
42	Consultant 2	-	1113	473845	94442	343512	76343	182659	43134	5.02	4.50	4.23	-	-
43	Consultant 2	-	9456	3785413	731174	2506718	503830	1248609	266210	5.18	4.98	4.69	-	-
44	Consultant 2	-	3345	1297834	287261	956024	216269	518307	131230	4.52	4.42	3.95	-	-
45	Consultant 2	-	4272	2488882	487262	1723321	363829	940757	216373	5.11	4.74	4.35	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
46	Consultant 4	2.5	16615	3564601	1550220	3076823	1007973	1698474	560539	2.30	3.05	3.03	16/09/2009	01/10/2009
47	Consultant 4	1	461	2081266	830691	2089487	565078	1045786	345112	2.51	3.70	3.03	16/09/2009	01/10/2009
48	Consultant 4	1.5	915	386474	118668	372124	84463	224409	50643	3.26	4.41	4.43	16/09/2009	01/10/2009
49	Consultant 4	0.3	367	132834	26456	108974	19714	62981	12534	5.02	5.53	5.02	01/10/2009	06/10/2009
50	Consultant 4	0.9	1117	394316	72446	305628	54375	155073	31074	5.44	5.62	4.99	01/10/2009	06/10/2009
51	Consultant 4	0.5	1752	701160	159399	605894	111396	360656	74134	4.40	5.44	4.86	01/10/2009	06/10/2009
52	Consultant 4	0.5	89	23309	5805	17520	4189	9201	2384	4.02	4.18	3.86	01/10/2009	06/10/2009
53	Consultant 4	0.2	84	16149	3384	12995	2620	7568	1780	4.77	4.96	4.25	01/10/2009	06/10/2009
54	Consultant 1	0	5339	3214180	776394	2531278	516535	1354124	296515	4.14	4.90	4.57	15/08/2009	07/10/2009
55	Consultant 1	1-1.5	6630	1107031	960176	876267	691303	655932	264097	1.15	1.27	2.48	15/08/2009	14/09/2009
56	Consultant 1	0-2	2143	668140	148163	529213	104925	312138	63853	4.51	5.04	4.89	Unknown	05/10/2009
57	Consultant 1	4-4.8	431	114809	33540	97060	25336	62004	16255	3.42	3.83	3.81	Unknown	05/10/2009
58	Consultant 1	3.5	571	180119	48441	137464	31423	76352	19241	3.72	4.37	3.97	Unknown	05/10/2009
59	Consultant 1	0-1.5	1825	602187	119190	480829	95660	289968	56747	5.05	5.03	5.11	Unknown	05/10/2009
60	Consultant 1	0-1	102	37325	8746	30112	6035	16342	4023	4.27	4.99	4.06	Validation Sample	-
61	Consultant 1	0-1	2759	911139	183152	622103	114178	305054	62540	4.97	5.45	4.88	Validation Sample	-
62	Consultant 1	0-1	5008	1680713	321869	1158685	212996	567814	112144	5.22	5.44	5.06	Validation Sample	-
63	Consultant 1	0-1	1551	433653	106820	323463	68549	171727	41405	4.06	4.72	4.15	Validation Sample	-
64	Consultant 4	1.5	383	16620	42129	59135	35800	49065	28455	0.39	1.65	1.72	01/11/2008	01/10/2009
65	Consultant 4	2.5	712	100193	64252	88526	63423	79858	49684	1.56	1.40	1.61	01/11/2008	01/10/2009
66	Consultant 4	2.5	1278	172441	151229	141344	128681	79380	79863	1.14	1.10	0.99	01/11/2008	01/10/2009
67	Consultant 4	0.1	21884	6857999	1456734	4897236	1077185	2526571	614668	4.71	4.55	4.11	27/09/2009	05/10/2009
68	Consultant 4	0.1	21860	2512383	1503683	2994069	1028736	1776458	498674	1.67	2.91	3.56	27/09/2009	05/10/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
69	Consultant 4	COMP	11870	2385648	791728	2219915	521963	1136976	245209	3.01	4.25	4.64	27/09/2009	05/10/2009
70	Consultant 5	1.7	124	47348	9525	36332	6774	20479	4681	4.97	5.36	4.37	01/10/2008	30/09/2009
71	Consultant 5	0.75	118	266343	172078	310595	125765	197335	79303	1.55	2.47	2.49	01/10/2008	30/09/2009
72	Consultant 5	0.1	12939	4515090	1002153	3541529	673001	1957394	418063	4.51	5.26	4.68	01/10/2008	30/09/2009
73	Consultant 5	0.8	161	33429	9525	32795	7490	23843	5705	3.51	4.38	4.18	01/10/2008	30/09/2009
74	Consultant 1	1-1.6	197	36215	19208	40120	13588	25911	9074	1.89	2.95	2.86	22/09/2009	07/10/2009
75	Consultant 1	0.4	8979	1306844	1049411	1205916	709671	710345	437230	1.25	1.70	1.62	22/09/2009	07/10/2009
76	Consultant 1	0.4-1.2	10284	4081266	830691	3089487	565078	1645786	345112	4.91	5.47	4.77	22/09/2009	07/10/2009
77	Consultant 1	0.4	8268	1108581	876902	2059815	546991	929010	256913	1.26	3.77	3.62	22/09/2009	07/10/2009
78	Consultant 1	1.6-1.9	12492	2913045	934525	2002916	591721	1022548	309575	3.12	3.38	3.30	22/09/2009	07/10/2009
79	Consultant 2	-	2314	1725005	349110	1294746	266121	749875	172693	4.94	4.87	4.34	-	-
80	Consultant 2	-	5996	3597317	671878	2695037	591229	1709330	413968	5.35	4.56	4.13	-	-
81	Consultant 2	-	11985	6373669	1703717	6919324	1995772	6153003	1724851	3.74	3.47	3.57	-	-
82	Consultant 2	-	12556	8058062	1958015	7059094	1810818	5474481	1352009	4.12	3.90	4.05	-	-
83	Consultant 2	-	10047	6834040	1424966	5129881	1196119	3124830	736200	4.80	4.29	4.24	-	-
84	Consultant 2	-	8621	3772395	864428	2444146	564380	1290535	334405	4.36	4.33	3.86	-	-
85	Consultant 2	-	3574	1766956	382318	1116882	279341	587491	150615	4.62	4.00	3.90	-	-
86	Consultant 2	-	149	32226	13285	25748	8869	13459	4382	2.43	2.90	3.07	-	-
87	Consultant 2	-	17653	6665270	1730909	4671152	1126706	2538971	654120	3.85	4.15	3.88	-	-
88	Consultant 2	-	10129	3983404	873077	2594092	598546	1307351	325008	4.56	4.33	4.02	-	-
89	Consultant 2	-	2521	1219464	208206	886259	132077	373290	91453	5.86	6.71	4.08	-	-
90	Consultant 2	-	8616	3349911	402189	2387808	357698	972466	122582	8.33	6.68	7.93	-	-
91	Consultant 2	-	4007	2226210	395738	1650540	246376	662575	95501	5.63	6.70	6.94	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
92	Consultant 4	-	58787	23690671	7573504	21462014	6995174	15448567	5153352	3.13	3.07	3.00	Unknown	08/10/2009
93	Consultant 1	3-3.5	2616	793577	148769	481700	87847	243748	53756	5.33	5.48	4.53	Unknown	08/10/2009
94	Consultant 1	1.9-3.9	4739	1098432	260297	862106	168308	550679	118737	4.22	5.12	4.64	Unknown	08/10/2009
95	Consultant 1	2.3	2336	817877	230799	668050	153265	390933	101742	3.54	4.36	3.84	Unknown	08/10/2009
96	Consultant 1	0.4	2432	786782	231085	488342	127442	249423	83577	3.40	3.83	2.98	14/10/2008	03/11/2008
97	Consultant 2	-	27633	8039029	1990772	6468158	1270078	3582097	739739	4.04	5.09	4.84	-	-
98	Consultant 2	-	892	205621	54838	173781	38971	97797	21523	3.75	4.46	4.54	-	-
99	Consultant 2	-	204	59849	14795	50667	10261	28248	5852	4.05	4.94	4.83	-	-
100	Consultant 2	-	3698	1476661	393714	1415618	300640	881276	172311	3.75	4.71	5.11	-	-
101	Consultant 1	1.5-2.5	77	18966	3820	12179	2781	6518	1668	4.96	4.38	3.91	Unknown	12/10/2009
102	Consultant 1	2.0-2.5	708	313637	54826	224967	55469	148243	34133	5.72	4.06	4.34	Unknown	12/10/2009
103	Consultant 1	0-0.4	1539	29098	15094	45205	19866	38709	18251	1.93	2.28	2.12	Validation Sample	-
104	Consultant 1	0.8-1.3	64	102321	24704	67424	17946	32911	11006	4.14	3.76	2.99	Validation Sample	-
105	Consultant 4	-	1281	292903	142238	336636	159414	261836	119586	2.06	2.11	2.19	08/10/2009	12/10/2009
106	Consultant 4	0.4	5893	579380	485929	498101	300504	318691	200498	1.19	1.66	1.59	01/09/2009	07/10/2009
107	Consultant 4	0.4	2733	509250	170550	332351	169228	319472	115639	2.99	1.96	2.76	01/09/2009	25/09/2009
108	Consultant 4	0.75	536	90285	21805	72980	18088	38959	9525	4.14	4.03	4.09	01/09/2009	07/10/2009
109	Consultant 4	0.5	1133	502378	313518	347724	294591	305686	183117	1.60	1.18	1.67	01/09/2009	07/10/2009
110	Consultant 4	0.9	1182	384651	107471	507121	136026	406774	93869	3.58	3.73	4.33	Validation Sample	-
111	Consultant 4	2.45	359	130116	17964	107464	17301	63699	11124	7.24	6.21	5.73	Validation Sample	-
112	Consultant 2	-	488	96643	40053	94484	30316	62867	22360	2.41	3.12	2.81	-	-
113	Consultant 1	0.6-1.0	150	63084	11389	52842	10808	33070	6509	5.54	4.89	5.08	29/09/2009	14/10/2009
114	Consultant 1	0	3120	1622128	263764	1141671	234603	722538	151905	6.15	4.87	4.76	29/09/2009	14/10/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
115	Consultant 1	0.1-0.3	483	244227	39728	174445	32649	99995	19352	6.15	5.34	5.17	29/09/2009	14/10/2009
116	Consultant 4	0.25	2365	611193	194396	518000	151070	308160	81308	3.14	3.43	3.79	06/10/2009	07/10/2009
117	Consultant 4	-	2365	938037	288495	791979	212743	474347	121035	3.25	3.72	3.92	06/10/2009	07/10/2009
118	Consultant 2	-	542	177135	38318	143322	27541	90698	20585	4.62	5.20	4.41	-	-
119	Consultant 2	-	389	80404	14151	54751	10193	27545	6302	5.68	5.37	4.37	-	-
120	Consultant 2	-	19518	5851872	914318	3708181	461539	1850142	294474	6.40	8.03	6.28	-	-
121	Consultant 2	-	255	21249	5951	17228	4179	9883	2848	3.57	4.12	3.47	-	-
122	Consultant 2	-	979	314415	62000	182672	32413	68161	15516	5.07	5.64	4.39	-	-
123	Consultant 2	-	298	96780	19383	53652	8675	17611	4098	4.99	6.18	4.30	-	-
124	Consultant 2	-	10439	3554825	719204	2019386	341128	715978	152090	4.94	5.92	4.71	-	-
125	Consultant 2	-	1702	936527	288617	775480	142599	298053	72422	3.24	5.44	4.12	-	-
126	Consultant 2	-	542	71016	17710	47310	12614	23243	8289	4.01	3.75	2.80	-	-
127	Consultant 2	-	<1	20038	6143	15329	3138	6295	1714	3.26	4.88	3.67	-	-
128	Consultant 2	-	389	174647	38986	128269	20821	56379	10436	4.48	6.16	5.40	-	-
129	Consultant 2	-	19518	107853	33330	89712	21010	52292	12045	3.24	4.27	4.34	-	-
130	Consultant 2	-	255	24110	12645	26168	8492	14910	5691	1.91	3.08	2.62	-	-
131	Consultant 2	-	<10	346037	111305	350519	100252	234180	73822	3.11	3.50	3.17	-	-
132	Consultant 6	1-1.5	1361	602172	166326	537578	108916	290243	64061	3.62	4.94	4.53	Unknown	13/10/2009
133	Consultant 6	1-1.5	3636	1302081	331424	1025121	205648	522788	117274	3.93	4.98	4.46	Unknown	13/10/2009
134	Consultant 6	1-1.5	537	136464	42011	110882	28883	61800	17290	3.25	3.84	3.57	01/10/2009	13/10/2009
135	Consultant 6	0.4	11814	4062206	797998	3135609	564096	1899997	391024	5.09	5.56	4.86	Unknown	15/10/2009
136	Consultant 6	0.4	3483	1375051	257393	1063490	187817	630075	122417	5.34	5.66	5.15	Unknown	15/10/2009
137	Consultant 1	1-1.3	1235	366817	45342	280058	39190	159713	24653	8.09	7.15	6.48	Unknown	15/10/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12} : iC_{14}$	$nC_{13} : iC_{15}$	$nC_{14} : iC_{16}$	Release Date	Sampling Date
138	Consultant 4	0.8	329	134577	32663	100910	17109	53225	10976	4.12	5.90	4.85	29/09/2009	12/10/2009
139	Consultant 4	0.2	1977	665903	185144	574324	118627	321191	71915	3.60	4.84	4.47	28/09/2009	12/10/2009
140	Consultant 4	0.2	576	83595	18748	60742	11807	32335	6511	4.46	5.14	4.97	24/06/2008	16/10/2009
141	Consultant 4	0.5	72	28804	7409	28435	5923	19210	4353	3.89	4.80	4.41	24/06/2008	16/10/2009
142	Consultant 4	0.6	620	79094	46091	87297	34627	59303	27787	1.72	2.52	2.13	24/06/2008	16/10/2009
143	Consultant 7	0.4	3918	1951337	506066	1777466	457378	1018163	227638	3.86	3.89	4.47	Unknown	19/10/2009
144	Consultant 7	0.5	255	19528	7291	19134	6448	12821	4296	2.68	2.97	2.98	Unknown	19/10/2009
145	Consultant 2	-	2639	761109	180325	615758	136592	342716	74008	4.22	4.51	4.63	-	-
146	Consultant 2	-	4313	1241093	279289	945086	211726	523404	102928	4.44	4.46	5.09	-	-
147	Consultant 2	-	1344	432483	108358	352119	80638	206498	43736	3.99	4.37	4.72	-	-
148	Consultant 2	-	1927	555971	66886	425616	92791	232226	51645	8.31	4.59	4.50	-	-
149	Consultant 2	-	1461	572445	167481	531403	137240	344959	81249	3.42	3.87	4.25	-	-
150	Consultant 2	-	3584	818239	204336	616456	144336	340705	80338	4.00	4.27	4.24	-	-
151	Consultant 2	-	8385	2588149	671886	2046742	473623	1189935	272537	3.85	4.32	4.37	-	-
152	Consultant 2	-	10365	2670215	682086	2044530	462126	1151528	268531	3.91	4.42	4.29	-	-
153	Consultant 4	0.25	491	57070	18319	50101	13371	27393	8516	3.12	3.75	3.22	12/09/2009	21/09/2009
154	Consultant 4	0.1	1447	702443	158543	598636	132537	323769	67856	4.43	4.52	4.77	12/09/2009	21/09/2009
155	Consultant 1	0.7-2.2	191	72950	20997	51573	11755	22756	6901	3.47	4.39	3.30	Unknown	20/10/2009
156	Consultant 1	0.7-2.2	183	74709	21551	55216	11622	24311	7121	3.47	4.75	3.41	Unknown	20/10/2009
157	Consultant 1	1.0-2.5	687	255871	63649	165999	30827	66669	18365	4.02	5.38	3.63	Unknown	20/10/2009
158	Consultant 1	1-2.1	447	143638	32623	85303	15181	33642	7878	4.40	5.62	4.27	Unknown	20/10/2009
159	Consultant 1	0-1.3	1390	532086	137225	433573	119864	257087	60951	3.88	3.62	4.22	Unknown	20/10/2009
160	Consultant 1	0-1.2	1065	363408	84032	294780	76003	174925	37264	4.32	3.88	4.69	Unknown	20/10/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
161	Consultant 2	-	275	92707	18158	69605	10930	36927	7487	5.11	6.37	4.93	-	-
162	Consultant 2	-	83	28637	4513	19015	2782	10201	1869	6.35	6.84	5.46	-	-
163	Consultant 2	-	1374	491168	101754	333245	61910	184735	41195	4.83	5.38	4.48	-	-
164	Consultant 2	-	1079	375428	52343	253982	34050	134677	23042	7.17	7.46	5.84	-	-
165	Consultant 2	-	2358	454364	97829	370929	81109	220680	64363	4.64	4.57	3.43	-	-
166	Consultant 2	-	266	30860	12753	28046	9317	17201	6827	2.42	3.01	2.52	-	-
167	Consultant 6	1	3103	1147508	233678	972446	207830	598354	139009	4.91	4.68	4.30	01/06/2009	22/10/2009
168	Consultant 4	0.2	438	25818	9501	24487	6498	14200	3806	2.72	3.77	3.73	01/07/2009	23/10/2009
169	Consultant 4	0-0.1	13081	3631903	1273516	2928104	816020	1501567	401292	2.85	3.59	3.74	01/07/2009	23/10/2009
170	Consultant 4	0	46794	12516877	4085262	9782814	2850304	4874413	1254451	3.06	3.43	3.89	01/07/2009	23/10/2009
171	Consultant 4	0.4	351	173280	42551	139893	27583	71041	16112	4.07	5.07	4.41	01/07/2009	23/10/2009
172	Consultant 2	-	1616	683926	150198	546314	89924	282616	49912	4.55	6.08	5.66	-	-
173	Consultant 2	-	8376	3823902	770250	2851211	439212	1338811	230953	4.96	6.49	5.80	-	-
174	Consultant 2	-	4613	2223785	711599	2837693	652371	2363014	623173	3.13	4.35	3.79	-	-
175	Consultant 2	-	4427	2086549	465784	1708699	274771	868752	162507	4.48	6.22	5.35	-	-
176	Consultant 2	-	5842	2755785	533810	1952194	258694	887147	148599	5.16	7.55	5.97	-	-
177	Consultant 2	-	2217	1009881	212209	775284	106881	361887	61153	4.76	7.25	5.92	-	-
178	Consultant 2	-	424	224670	70296	239453	45562	138219	28207	3.20	5.26	4.90	-	-
179	Consultant 2	-	20046	8656051	2216549	6258944	1254907	3241849	742071	3.91	4.99	4.37	-	-
180	Consultant 2	-	41393	15475429	5609517	14913922	3676034	9326189	2337051	2.76	4.06	3.99	-	-
181	Consultant 2	-	569	240816	35461	151446	21624	80290	14310	6.79	7.00	5.61	-	-
182	Consultant 2	-	34045	12530408	3475158	10029936	2454490	5929731	1421470	3.61	4.09	4.17	-	-
183	Consultant 2	-	15396	6135117	1686976	4877327	1176187	2640574	694087	3.64	4.15	3.80	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12} : iC_{14}$	$nC_{13} : iC_{15}$	$nC_{14} : iC_{16}$	Release Date	Sampling Date
184	Consultant 2	-	12457	5394059	1366027	4348444	925472	2385682	519323	3.95	4.70	4.59	-	-
185	Consultant 2	-	3859	1906827	488548	1632176	345815	885183	209279	3.90	4.72	4.23	-	-
186	Consultant 5	1	1334	258659	57257	238550	47649	163957	35843	4.52	5.01	4.57	Unknown	26/10/2009
187	Consultant 5	1	4689	1074291	313065	760517	232360	420383	160840	3.43	3.27	2.61	Unknown	26/10/2009
188	Consultant 5	1	110	31886	9238	28700	6609	17207	4426	3.45	4.34	3.89	Unknown	26/10/2009
189	Consultant 5	2	100	43147	11460	35752	7600	20642	5130	3.77	4.70	4.02	Unknown	26/10/2009
190	Consultant 5	-	42	15026	4607	13090	3048	7867	2195	3.26	4.29	3.58	Unknown	26/10/2009
191	Consultant 4	0.1	1695	495555	99621	388257	69458	229926	46028	4.97	5.59	5.00	24/10/2009	27/10/2009
192	Consultant 4	0.3	716	946454	202653	800831	141892	495709	100287	4.67	5.64	4.94	24/10/2009	27/10/2009
193	Consultant 4	0.5	1848	199783	57953	204140	40702	126870	28388	3.45	5.02	4.47	24/10/2009	27/10/2009
194	Consultant 4	0.25	<30	429026	175335	572054	132058	335355	91841	2.45	4.33	3.65	01/11/2009	16/11/2009
195	Consultant 4	0.55	141	2145007	402725	1461563	245295	706741	147674	5.33	5.96	4.79	01/11/2009	16/11/2009
196	Consultant 1	-	103372	8549831	1806768	6579320	1438768	3787289	918467	4.73	4.57	4.12	Water Sample	29/10/2009
197	Consultant 1	0.9-2.9	<30	13583	2933	11629	2297	6999	1748	4.63	5.06	4.00	06/12/2008	29/10/2009
198	Consultant 1	0.8-2.0	68	30506	5605	23217	4843	12967	2993	5.44	4.79	4.33	06/12/2008	29/10/2009
199	Consultant 1	0.8-1.8	1673	743173	130983	552760	113124	302722	62292	5.67	4.89	4.86	06/12/2008	29/10/2009
200	Consultant 1	-	4960	408261	110485	462325	94140	310130	60602	3.70	4.91	5.12	Water Sample	29/10/2009
201	Consultant 4	0.5	18734	6897745	1142402	6108512	1022447	3792272	579358	6.04	5.97	6.55	29/10/2009	30/10/2009
202	Consultant 4	0.75	9233	3967183	601735	2938911	524768	1910536	311599	6.59	5.60	6.13	29/10/2009	30/10/2009
203	Consultant 4	0.1	6988	2589829	401066	1842892	340644	975538	199247	6.46	5.41	4.90	29/10/2009	30/10/2009
204	Consultant 4	0.1	5743	1244742	227402	858933	184634	437092	96589	5.47	4.65	4.53	26/10/2009	27/10/2009
205	Consultant 2	-	474	67834	13535	42579	7738	20255	4950	5.01	5.50	4.09	-	-
206	Consultant 2	-	1412	323089	63756	203363	33211	94382	20821	5.07	6.12	4.53	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
207	Consultant 2	-	251	36304	16131	34613	8799	20084	5964	2.25	3.93	3.37	-	-
208	Consultant 2	-	2860	759411	147716	477144	76476	218544	46893	5.14	6.24	4.66	-	-
209	Consultant 2	-	879	220775	47851	147300	24299	72012	15567	4.61	6.06	4.63	-	-
210	Consultant 2	-	1954	459011	163264	314803	77763	123358	38117	2.81	4.05	3.24	-	-
211	Consultant 2	-	1553	319299	146924	241857	69524	97170	35642	2.17	3.48	2.73	-	-
212	Consultant 2	-	1085	186561	118139	130228	53257	52682	27270	1.58	2.45	1.93	-	-
213	Consultant 2	-	1202	439366	113148	273466	52033	111765	24738	3.88	5.26	4.52	-	-
214	Consultant 2	-	3747	1419993	338171	919443	154724	333174	76635	4.20	5.94	4.35	-	-
215	Consultant 4	0.45	8113	1861239	545202	1421934	411465	791434	227086	3.41	3.46	3.49		14/10/2009
216	Consultant 4	0.85	1506	546917	151536	476928	108279	277244	63577	3.61	4.40	4.36		14/10/2009
217	Consultant 4	-	6861	1399391	440497	1061420	320368	626734	181517	3.18	3.31	3.45		14/10/2009
218	Consultant 2	-	7245	2272870	523746	1778358	368279	1061124	215018	4.34	4.83	4.94	-	-
219	Consultant 2	-	3078	871822	138571	653924	113742	491661	80441	6.29	5.75	6.11	-	-
220	Consultant 2	-	12801	3988323	857280	3018685	611366	1788178	357874	4.65	4.94	5.00	-	-
221	Consultant 2	-	2414	60107	48458	87957	42674	70285	32672	1.24	2.06	2.15	-	-
222	Consultant 1	0-1.3	2119	764520	144326	531360	79883	227347	41117	5.30	6.65	5.53	Unknown	30/10/2009
223	Consultant 1	0-1.5	600	242768	51127	193476	29741	86759	18002	4.75	6.51	4.82	Unknown	30/10/2009
224	Consultant 1	0-1.9	83	24166	5291	17880	3091	7799	1829	4.57	5.78	4.26	Unknown	30/10/2009
225	Consultant 1	0-1.6	562	177976	43316	149723	25741	70974	15196	4.11	5.82	4.67	Unknown	30/10/2009
226	Consultant 1	0-2	294	75056	15860	57514	9816	26855	6038	4.73	5.86	4.45	Unknown	30/10/2009
227	Consultant 2	-	4161	1340730	274891	1029392	193668	544571	118533	4.88	5.32	4.59	-	-
228	Consultant 2	-	2415	795665	166088	609725	118063	328787	70468	4.79	5.16	4.67	-	-
229	Consultant 2	-	13773	5004263	1036438	3744059	681484	1953603	397278	4.83	5.49	4.92	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12} : iC_{14}$	$nC_{13} : iC_{15}$	$nC_{14} : iC_{16}$	Release Date	Sampling Date
230	Consultant 2	-	747	188640	37941	143805	26509	75251	16257	4.97	5.42	4.63	-	-
231	Consultant 2	-	24140	6241079	1283264	4752547	904016	2585462	521745	4.86	5.26	4.96	-	-
232	Consultant 2	-	7167	2370410	482399	1770374	307229	891492	167985	4.91	5.76	5.31	-	-
233	Consultant 2	-	5856	1820109	391513	1407806	247377	706628	143598	4.65	5.69	4.92	-	-
234	Consultant 2	-	965	84112	22900	60418	14669	31193	8344	3.67	4.12	3.74	-	-
235	Consultant 2	-	1614	345031	96339	292654	59203	145495	32617	3.58	4.94	4.46	-	-
236	Consultant 1	0.1	2827	1317300	255518	946388	151759	490423	95986	5.16	6.24	5.11	Unknown	02/11/2009
237	Consultant 1	0	2411	981606	182930	636835	93577	304669	52882	5.37	6.81	5.76	Unknown	02/11/2009
238	Consultant 4	0.2-0.35	690	100819	18034	67725	11995	34703	7195	5.59	5.65	4.82	18/10/2009	04/11/2009
239	Consultant 4	0.8	264	47196	10703	38862	8325	22615	5160	4.41	4.67	4.38	18/10/2009	04/11/2009
240	Consultant 4	1	1614	458349	118229	336325	76973	158635	52710	3.88	4.37	3.01	18/10/2009	30/10/2009
241	Consultant 4	0.2	4117	1384475	448940	1285706	312624	821502	205227	3.08	4.11	4.00	14/10/2009	16/10/2009
242	Consultant 4	0.4	6718	2566811	579677	1953380	356621	1339520	232708	4.43	5.48	5.76	14/10/2009	16/10/2009
243	Consultant 4	0.4	2725	1546396	336575	1271351	230051	888382	160303	4.59	5.53	5.54	14/10/2009	16/10/2009
244	Consultant 4	0.5	1684	682301	152513	614816	110464	436671	70633	4.47	5.57	6.18	14/10/2009	16/10/2009
245	Consultant 4	0.5	5442	2039731	466905	1539706	311286	1085834	208414	4.37	4.95	5.21	14/10/2009	16/10/2009
246	Consultant 2	-	974	298402	55121	188827	35490	82731	18272	5.41	5.32	4.53	-	-
247	Consultant 2	-	862	266377	49921	166786	32446	71763	15779	5.34	5.14	4.55	-	-
248	Consultant 2	-	131	36477	8425	27574	6053	13420	3295	4.33	4.56	4.07	-	-
249	Consultant 2	-	5957	2228561	479990	1540608	261190	676723	152829	4.64	5.90	4.43	-	-
250	Consultant 2	-	5885	1752974	322622	1062014	192038	428740	86593	5.43	5.53	4.95	-	-
251	Consultant 2	-	735	240966	48289	152041	27742	61143	12386	4.99	5.48	4.94	-	-
252	Consultant 2	-	144	49816	11756	41645	9495	24306	6274	4.24	4.39	3.87	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
253	Consultant 2	-	38	12388	2873	7965	1611	2877	912	4.31	4.94	3.15	-	-
254	Consultant 2	-	4551	418467	108575	310013	61701	149023	40829	3.85	5.02	3.65	-	-
255	Consultant 1	0	20411	1073173	191842	860756	137137	447084	83673	5.59	6.28	5.34	10/10/2009	03/11/2009
256	Consultant 1	0.4	2955	615604	124333	357134	93275	135963	50806	4.95	3.83	2.68	10/10/2009	03/11/2009
257	Consultant 1	0.5	5735	541949	413428	420280	329134	336264	206910	1.31	1.28	1.63	01/09/2009	07/10/2009
258	Consultant 1	0.4	2969	1550323	485007	1470491	368948	566514	213724	3.20	3.99	2.65	10/10/2009	10/11/2009
259	Consultant 2	-	3135	985687	208438	875611	194425	592730	148202	4.73	4.50	4.00	-	-
260	Consultant 2	-	6705	2409575	376301	1745529	303774	967498	181327	6.40	5.75	5.34	-	-
261	Consultant 2	-	2452	910410	140511	654037	119457	349903	70371	6.48	5.48	4.97	-	-
262	Consultant 2	-	713	329902	55081	248145	43301	139963	27223	5.99	5.73	5.14	-	-
263	Consultant 2	-	1315	513133	87418	381023	70743	214573	42761	5.87	5.39	5.02	-	-
264	Consultant 2	-	2786	989865	133421	754673	107037	442157	77356	7.42	7.05	5.72	-	-
265	Consultant 2	-	211	90281	12586	67603	9487	38383	6877	7.17	7.13	5.58	-	-
266	Consultant 2	-	187	57465	8035	42079	6344	23326	4239	7.15	6.63	5.50	-	-
267	Consultant 2	-	465	172754	28048	152801	24634	94873	17652	6.16	6.20	5.37	-	-
268	Consultant 2	-	1885	695593	87065	494669	67335	273472	43892	7.99	7.35	6.23	-	-
269	Consultant 2	-	250	81688	10984	58838	8864	33171	5802	7.44	6.64	5.72	-	-
270	Consultant 2	-	7912	2438036	604196	2280190	459335	1412795	318969	4.04	4.96	4.43	-	-
271	Consultant 2	-	906	140480	44395	178239	48733	155675	42929	3.16	3.66	3.63	-	-
272	Consultant 2	-	14842	7536540	2314805	8415142	2052349	5892438	1445971	3.26	4.10	4.08	-	-
273	Consultant 2	-	44334	16511252	6749420	21902478	6512584	17522298	4959089	2.45	3.36	3.53	-	-
274	Consultant 1	0.5	5857	1310194	376036	961565	274885	457089	140859	3.48	3.50	3.25	Unknown	11/11/2009
275	Consultant 1	0.9-0.1	13734	2760444	972390	1518441	651392	904644	341348	2.84	2.33	2.65	16/09/2009	05/10/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12} : iC_{14}$	$nC_{13} : iC_{15}$	$nC_{14} : iC_{16}$	Release Date	Sampling Date
276	Consultant 1	0.2	465	92779	37906	72090	25335	40943	16653	2.45	2.85	2.46	16/09/2009	10/11/2009
277	Consultant 1	7.5	355332	21179628	5245152	13415377	2756260	5107329	1068597	4.04	4.87	4.78	Water Sample	-
278	Consultant 2	-	3865	879127	252892	552211	128993	236576	70058	3.48	4.28	3.38	-	-
279	Consultant 2	-	2507	860757	174204	512135	92167	207312	50349	4.94	5.56	4.12	-	-
280	Consultant 2	-	754	117779	57745	123883	33507	71654	20947	2.04	3.70	3.42	-	-
281	Consultant 2	-	7285	1983309	446250	1345843	239727	598975	122404	4.44	5.61	4.89	-	-
282	Consultant 2	-	7228	2147992	478559	1295147	240280	525504	128000	4.49	5.39	4.11	-	-
283	Consultant 2	-	13683	4053488	767237	2469890	442266	1038139	236076	5.28	5.58	4.40	-	-
284	Consultant 2	-	3118	1243626	230276	920317	163601	493891	104054	5.40	5.63	4.75	-	-
285	Consultant 2	-	3204	1364201	257881	1026863	178096	564494	116022	5.29	5.77	4.87	-	-
286	Consultant 2	-	19714	6264408	1476163	4938341	1050769	2860991	710348	4.24	4.70	4.03	-	-
287	Consultant 2	-	3954	3502332	675697	2659972	492854	1467790	297051	5.18	5.40	4.94	-	-
288	Consultant 2	-	11601	3326086	865058	2629604	565609	1456466	336128	3.84	4.65	4.33	-	-
289	Consultant 2	-	7855	2305923	467726	1656891	311355	807810	162955	4.93	5.32	4.96	-	-
290	Consultant 2	-	136	38895	19536	45010	14243	31894	9440	1.99	3.16	3.38	-	-
291	Consultant 2	-	112	204833	84842	203017	52216	114524	28943	2.41	3.89	3.96	-	-
292	Consultant 8	1.5	315	66752	14833	41648	8337	22722	5553	4.50	5.00	4.09	Unknown	16/11/2009
293	Consultant 8	0.8	623	241598	45074	150341	24386	84071	16796	5.36	6.17	5.01	Unknown	16/11/2009
294	Consultant 8	1	207	329626	60528	202266	35406	115370	24659	5.45	5.71	4.68	Unknown	16/11/2009
295	Consultant 4	0.4	144	49740	11892	38262	8385	21515	6599	4.18	4.56	3.26	Unknown	17/11/2009
296	Consultant 4	0.2	530	254618	56342	213520	39672	129527	25149	4.52	5.38	5.15	Unknown	17/11/2019
297	Consultant 4	2.5	10980	4523823	1170486	3805141	1134460	2394593	635867	3.86	3.35	3.77	Validation Sample	-
298	Consultant 1	0.6 - 2.0	282	101457	30712	90433	20179	48631	12138	3.30	4.48	4.01	Unknown	17/11/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
299	Consultant 1	0.7 - 2.0	983	460322	99640	355751	69787	175299	37673	4.62	5.10	4.65	Unknown	17/11/2009
300	Consultant 1	0.8 - 2.0	1413	642772	133944	484487	104155	244225	52419	4.80	4.65	4.66	Unknown	17/11/2009
301	Consultant 4	0.3	225	55695	12945	41747	9187	17394	3906	4.30	4.54	4.45	09/11/2009	13/11/2009
302	Consultant 4	0.25	475	162433	48741	155422	37409	77710	18582	3.33	4.15	4.18	09/11/2009	13/11/2009
303	Consultant 4		107	37108	8641	26932	5374	11685	2904	4.29	5.01	4.02	09/11/2009	13/11/2009
304	Consultant 4	0.75	112	47520	7258	32758	4582	18880	3518	6.55	7.15	5.37	01/11/2009	16/11/2009
305	Consultant 4	Surface	59797	10928657	2134313	9376522	1453645	6836068	1327592	5.12	6.45	5.15	01/11/2009	16/11/2009
306	Consultant 4	-	507	213460	28838	140680	16953	76375	11795	7.40	8.30	6.48	01/11/2009	16/11/2009
307	Consultant 4	0.5	731	1014506	219883	756642	134087	371460	84887	4.61	5.64	4.38	01/11/2009	16/11/2009
308	Consultant 4	0.25	141	61843	8582	38080	4670	19600	3139	7.21	8.15	6.24	01/11/2009	16/11/2009
309	Consultant 4	-	1009	415455	51373	250642	33326	136630	23763	8.09	7.52	5.75	01/11/2009	16/11/2009
310	Consultant 4	0.7	705	199319	41457	146741	28782	78643	19170	4.81	5.10	4.10	18/06/2009	09/11/2009
311	Consultant 4	0.7	99	21676	6222	19074	4071	10679	3022	3.48	4.69	3.53	18/06/2009	09/11/2009
312	Consultant 4	0.8	256	47115	9486	35705	6554	21555	4771	4.97	5.45	4.52	18/06/2009	09/11/2009
313	Consultant 4	0.4	441	20052	9048	22716	6713	18064	5990	2.22	3.38	3.02	18/06/2009	09/11/2009
314	Consultant 4	0.75	584	177271	25104	118315	17028	66244	12324	7.06	6.95	5.38	18/06/2009	09/11/2009
315	Consultant 4	-	1027	358039	49106	241772	34521	144012	27404	7.29	7.00	5.26	18/06/2009	09/11/2009
316	Consultant 1	-	1337	232953	109859	241372	88828	160859	56748	2.12	2.72	2.83	Unknown	20/11/2009
317	Consultant 2	-	130	18284	4843	13613	2878	6230	1631	3.78	4.73	3.82	-	-
318	Consultant 2	-	47	15469	3681	11409	2261	5400	1307	4.20	5.05	4.13	-	-
319	Consultant 2	-	6205	1723613	413432	1274392	220786	570980	110225	4.17	5.77	5.18	-	-
320	Consultant 2	-	2089	618157	130025	424801	74173	184601	36935	4.75	5.73	5.00	-	-
321	Consultant 2	-	10944	1945837	545312	1355035	278803	589556	140013	3.57	4.86	4.21	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
322	Consultant 2	-	42697	13910181	3465038	10733316	2221143	5774176	1266335	4.01	4.83	4.56	-	-
323	Consultant 2	-	4865	1327083	289157	932638	161793	398186	76251	4.59	5.76	5.22	-	-
324	Consultant 2	-	16692	4220142	999034	3150555	651687	1623490	357270	4.22	4.83	4.54	-	-
325	Consultant 2	-	642	232862	48893	178526	35888	98160	21122	4.76	4.97	4.65	-	-
326	Consultant 2	-	15727	3678378	1102148	2707793	771162	1482375	430571	3.34	3.51	3.44	-	-
327	Consultant 2	-	237	70948	23082	62143	15021	33076	8368	3.07	4.14	3.95	-	-
328	Consultant 2	-	4679	1233685	300943	919178	185597	452482	91982	4.10	4.95	4.92	-	-
329	Consultant 2	-	1501	473484	108572	365180	77838	200466	44828	4.36	4.69	4.47	-	-
330	Consultant 4	-	276	34288	14539	33479	11283	21147	7322	2.36	2.97	2.89	Validation Sample	-
331	Consultant 4	-	233	19609	6583	20652	5577	12314	3456	2.98	3.70	3.56	Validation Sample	-
332	Consultant 4	-	152	15891	6062	15689	4747	8957	3178	2.62	3.31	2.82	Validation Sample	-
333	Consultant 4	-	5910	1477784	287576	1094670	226291	637585	122352	5.14	4.84	5.21	21/10/2009	13/11/2009
334	Consultant 4	0.2	1841	468071	90650	353428	72755	202811	39108	5.16	4.86	5.19	21/10/2009	13/11/2009
335	Consultant 4	-	51	12529	3326	11140	2421	5991	1551	3.77	4.60	3.86	Validation Sample	-
336	Consultant 4	-	143	4329	1204	3645	1080	1963	630	3.60	3.38	3.12	Validation Sample	-
337	Consultant 4	-	<30	12014	3072	10768	2525	5947	1545	3.91	4.26	3.85	Validation Sample	-
338	Consultant 4	0.4	4841	1220162	379015	987446	263845	581677	145312	3.22	3.74	4.00	08/11/2009	13/11/2009
339	Consultant 4	0.8	3668	1216867	314773	986897	210852	529816	120638	3.87	4.68	4.39	08/11/2009	13/11/2009
340	Consultant 1	-	593	133428	43710	107469	30441	55923	17317	3.05	3.53	3.23	Validation Sample	-
341	Consultant 1	-	241	14174	9441	14424	6796	9209	4138	1.50	2.12	2.23	Validation Sample	-
342	Consultant 9	1	38990	7207373	2322597	4592346	1927177	2212749	1024084	3.10	2.38	2.16	Unknown	26/11/2009
343	Consultant 4	-	857	252633	53956	192958	39393	102880	22185	4.68	4.90	4.64	24/11/2009	27/11/2009
344	Consultant 4	0.7	2568	567388	144104	456376	107492	283553	58092	3.94	4.25	4.88	24/11/2009	27/11/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
345	Consultant 4	0.4	7823	1361430	422081	1647089	331389	778119	175670	3.23	4.97	4.43	24/11/2009	27/11/2009
346	Consultant 4	0.8	5119	1088941	263423	833134	193707	437590	105414	4.13	4.30	4.15	24/11/2009	27/11/2009
347	Consultant 4	0.8	0	4219	2141	4880	1740	3400	1157	1.97	2.80	2.94	24/11/2009	27/11/2009
348	Consultant 4	0.5	413	118646	28363	105333	24757	53512	13122	4.18	4.25	4.08	12/11/2009	01/12/2009
349	Consultant 4	0.5	10170	3279873	747747	2471031	543559	1205984	258531	4.39	4.55	4.66	12/11/2009	01/12/2009
350	Consultant 4	0.5	210	6776	1972	6203	1892	4549	1505	3.44	3.28	3.02	26/11/2009	30/11/2009
351	Consultant 4	0.1	7260	1998677	310920	1386266	286210	893079	178576	6.43	4.84	5.00	26/11/2009	30/11/2009
352	Consultant 4	1	1025	263454	43430	186474	40810	120464	27131	6.07	4.57	4.44	26/11/2009	30/11/2009
353	Consultant 2	-	786	172717	39702	130179	22560	59392	12103	4.35	5.77	4.91	-	-
354	Consultant 4	0.75	175	18420	6490	20951	7200	11769	2948	2.84	2.91	3.99	Validation Sample	-
355	Consultant 1	-	415657	23770131	7388197	21181291	5345386	12634133	2590119	3.22	3.96	4.88	Water Sample	-
356	Consultant 2	-	14761	3793734	965590	3112761	799642	1715065	455573	3.93	3.89	3.76	-	-
357	Consultant 2	-	2074	436151	146054	381173	121585	215727	75673	2.99	3.14	2.85	-	-
358	Consultant 2	-	7967	2313513	600696	1951527	516137	1079718	284518	3.85	3.78	3.79	-	-
359	Consultant 2	-	2054	925259	198800	741231	138940	352562	75964	4.65	5.33	4.64	-	-
360	Consultant 2	-	81945	22570951	5315423	15722365	3530718	7291001	1471893	4.25	4.45	4.95	-	-
361	Consultant 2	-	81945	3746572	707932	2467199	423214	1011278	171328	5.29	5.83	5.90	-	-
362	Consultant 2	-	1733	978999	190370	680983	109255	278641	53583	5.14	6.23	5.20	-	-
363	Consultant 2	-	373	118068	27211	97363	19771	48699	10797	4.34	4.92	4.51	-	-
364	Consultant 2	-	7877	3461190	729179	2817860	523641	1318994	263668	4.75	5.38	5.00	-	-
365	Consultant 2	-	2812	747021	197866	607804	153953	329712	89819	3.78	3.95	3.67	-	-
366	Consultant 2	-	440	143672	29741	109694	22747	53635	11178	4.83	4.82	4.80	-	-
367	Consultant 2	-	1567	500180	108500	401288	89723	214636	42980	4.61	4.47	4.99	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
368	Consultant 2	-	1803	605139	140975	502224	111014	271983	56171	4.29	4.52	4.84	-	-
369	Consultant 2	-	1117	395642	87705	320057	68873	176549	36987	4.51	4.65	4.77	-	-
370	Consultant 2	-	124	42645	9396	31558	6443	13692	2777	4.54	4.90	4.93	-	-
371	Consultant 2	-	833	27173849	7892839	21776032	5610184	12692105	3087545	3.44	3.88	4.11	-	-
372	Consultant 2	-	2523	1107130	257193	910349	194937	440897	88458	4.30	4.67	4.98	-	-
373	Consultant 2	-	4554	1669306	376883	1311351	286882	614434	137997	4.43	4.57	4.45	-	-
374	Consultant 4	0.8	1288	421928	86891	331277	61959	178431	35861	4.86	5.35	4.98	Validation Sample	07/12/2009
375	Consultant 4	0.8	1159	370941	73028	283050	50651	152104	30350	5.08	5.59	5.01	Validation Sample	07/12/2009
376	Consultant 4	1.2	142	38763	9655	33088	6757	17594	4068	4.01	4.90	4.32	Validation Sample	07/12/2009
377	Consultant 4	0.2-0.3	10964	2515424	443627	1846927	308454	939230	169220	5.67	5.99	5.55	03/12/2009	07/12/2009
378	Consultant 4	0.2	7015	2074753	394144	1575761	261351	832758	161094	5.26	6.03	5.17	03/12/2009	07/12/2009
379	Consultant 4	0.4	33	12727	2778	8271	1462	3476	822	4.58	5.66	4.23	03/12/2009	07/12/2009
380	Consultant 4	-	184	38985	16536	29192	13462	15275	7033	2.36	2.17	2.17	Validation Sample	-
381	Consultant 4	1.5	636	179003	38256	162137	39383	129572	29678	4.68	4.12	4.37	Validation Sample	-
382	Consultant 4	1.2	399	123954	20730	108320	16710	59192	9952	5.98	6.48	5.95	Validation Sample	-
383	Consultant 4	1.4	5646	1859784	371963	1365389	242345	648984	122156	5.00	5.63	5.31	Validation Sample	-
384	Consultant 4	0.5	102	37686	10631	33082	7000	16622	3755	3.54	4.73	4.43	Validation Sample	-
385	Consultant 4	0.8	685	118985	70307	195093	54377	154996	41726	1.69	3.59	3.71	Validation Sample	-
386	Consultant 4	2.2	17695	6247961	1171104	4388942	783791	2143348	421013	5.34	5.60	5.09	Validation Sample	-
387	Consultant 1	0.6-0.7	4512	623857	348116	463310	244120	302993	138800	1.79	1.90	2.18	01/11/2009	30/11/2009
388	Consultant 1	0.7-1.0	993	171041	80320	171685	85851	157612	60272	2.13	2.00	2.62	01/11/2009	14/12/2009
389	Consultant 1	0.7-1.0	141	42455	13026	36044	8203	19037	4933	3.26	4.39	3.86	01/11/2009	14/12/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
390	Consultant 1	-	1791	79967	36880	86032	33925	80155	26427	2.17	2.54	3.03	Water Sample	-
391	Consultant 1	0-1	215	22162	8895	21306	7754	14974	5034	2.49	2.75	2.97	Unknown	10/12/2009
392	Consultant 1	0-2	735	199866	59269	174618	42374	102550	27162	3.37	4.12	3.78	Unknown	10/12/2009
393	Consultant 1	5	12571	428564	120896	405942	108063	290859	79001	3.54	3.76	3.68	Unknown	10/12/2009
394	Consultant 1	0.2-2	181	26317	7012	23448	5946	15506	4941	3.75	3.94	3.14	Unknown	11/12/2009
395	Consultant 1	0.5	258	51729	15648	52742	13601	40730	12867	3.31	3.88	3.17	30/11/2009	11/12/2009
396	Consultant 1	0.4	984	349991	81224	291947	64243	152665	31939	4.31	4.54	4.78	30/11/2009	11/12/2009
397	Consultant 1	0.5-2.5	425	162066	34120	137079	29583	87855	19234	4.75	4.63	4.57	Unknown	09/12/2009
398	Consultant 2	-	3265		215834	1109822	153906	630345	94161	0.00	7.21	6.69	-	-
399	Consultant 2	-	6179	2351759	347215	1815394	245245	1002162	160554	6.77	7.40	6.24	-	-
400	Consultant 2	-	6942	2413215	346620	1802230	240994	1025476	153940	6.96	7.48	6.66	-	-
401	Consultant 2	-	1186	447373	72659	379390	53387	221412	36856	6.16	7.11	6.01	-	-
402	Consultant 2	-	205	85704	49632	117677	49755	108110	39490	1.73	2.37	2.74	-	-
403	Consultant 1	0-1	1044	234210	70014	179709	46471	86041	23011	3.35	3.87	3.74	Unknown	14/12/2009
404	Consultant 1	1.0-2.0	1119	244441	68222	183389	45958	84385	22914	3.58	3.99	3.68	Unknown	14/12/2009
405	Consultant 2	-	2862	1175470	214042	835641	156231	403619	64895	5.49	5.35	6.22	-	-
406	Consultant 2	-	2116	966403	185401	727690	134448	348297	65163	5.21	5.41	5.35	-	-
407	Consultant 2	-	1361	614273	120106	471733	90373	230180	44067	5.11	5.22	5.22	-	-
408	Consultant 2	-	15628	4563195	945575	3151078	624765	1336777	313095	4.83	5.04	4.27	-	-
409	Consultant 2	-	18988	5699416	1216623	3984025	845087	1675433	387207	4.68	4.71	4.33	-	-
410	Consultant 2	-	23630	6353172	1367271	4409868	923275	1866812	429137	4.65	4.78	4.35	-	-
411	Consultant 2	-	61	29236	13016	34870	10160	18499	5077	2.25	3.43	3.64	-	-
412	Consultant 2	-	2204	1485574	459968	1280137	289869	574165	130730	3.23	4.42	4.39	-	-

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12} : iC_{14}$	$nC_{13} : iC_{15}$	$nC_{14} : iC_{16}$	Release Date	Sampling Date
413	Consultant 2	-	5145	1824563	309364	1211988	186809	577645	116529	5.90	6.49	4.96	-	-
414	Consultant 2	-	8297	2638238	469932	1709011	286940	812450	158719	5.61	5.96	5.12	-	-
415	Consultant 2	-	192651	10973339	2056058	7477486	1285702	3810774	839208	5.34	5.82	4.54	-	-
416	Consultant 2	-	2261	852549	139098	559884	90176	275834	51295	6.13	6.21	5.38	-	-
417	Consultant 1	-	1730	674093	109793	387228	68730	181573	35428	6.14	5.63	5.13	13/11/2009	17/12/2009
418	Consultant 4	0.6	62	16143	3410	12414	2279	5385	1210	4.73	5.45	4.45	Validation Sample	-
419	Consultant 4	-	1945	759473	154577	618414	108330	337482	67676	4.91	5.71	4.99	Validation Sample	-
420	Consultant 1	-	636	83970	22592	66855	17192	38448	13846	3.72	3.89	2.78	Water Sample	-
421	Consultant 1	0.7-1.9	464	141553	35773	89384	18296	32313	9203	3.96	4.89	3.51	Unknown	16/12/2009
422	Consultant 1	1	314	107227	19971	74984	12587	31568	5326	5.37	5.96	5.93	Unknown	18/12/2009
423	Consultant 2	-	939	237199	55313	194676	40712	90599	21473	4.29	4.78	4.22	-	-
424	Consultant 2	-	1563	628896	139590	482998	89929	209304	48929	4.51	5.37	4.28	-	-
425	Consultant 2	-	757	232217	48984	170925	36474	74539	18301	4.74	4.69	4.07	-	-
426	Consultant 2	-	2200	771831	172132	587246	114665	264842	62868	4.48	5.12	4.21	-	-
427	Consultant 2	-	4034	1673194	634895	2267934	684424	1838903	512505	2.64	3.31	3.59	-	-
428	Consultant 2	-	7814	2807781	778828	2372895	580073	1396774	318195	3.61	4.09	4.39	-	-
429	Consultant 2	-	4022	1584007	434059	1363648	346454	802004	194997	3.65	3.94	4.11	-	-
430	Consultant 2	-	860	374191	122909	380745	99355	235255	55315	3.04	3.83	4.25	-	-
431	Consultant 2	-	3676	1557211	444752	1439555	354160	867273	207426	3.50	4.06	4.18	-	-
432	Consultant 2	-	291	129232	29293	95034	20096	41503	10724	4.41	4.73	3.87	-	-
433	Consultant 2	-	4130	1745305	347693	1183369	219992	505828	119876	5.02	5.38	4.22	-	-
434	Consultant 2	-	2058	745720	162786	555667	107580	235822	53027	4.58	5.17	4.45	-	-
435	Consultant 4	0.5	2724	1067731	300312	889594	164807	454399	94227	3.56	5.40	4.82	28/11/2009	03/12/2009

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	$nC_{12}:iC_{14}$	$nC_{13}:iC_{15}$	$nC_{14}:iC_{16}$	Release Date	Sampling Date
436	Consultant 4	0.2	942	407742	54453	355443	47762	181869	33780	7.49	7.44	5.38	29/11/2009	04/12/2009
437	Consultant 4	0.5	1445	340328	72605	358783	63170	193451	41873	4.69	5.68	4.62	29/11/2009	04/12/2009
438	Consultant 4	0.4	2330	1028138	291893	715029	179386	344451	106310	3.52	3.99	3.24	29/11/2009	09/12/2009
439	Consultant 4	0.65	10481	4613139	1078588	3681708	794214	1967297	529264	4.28	4.64	3.72	01/12/2009	11/12/2009
440	Consultant 4	0.65	4578	1478940	435096	1097761	279607	588346	178716	3.40	3.93	3.29	01/12/2009	11/12/2009
441	Consultant 4	0.35	2691	447132	223652	424643	147138	265799	99042	2.00	2.89	2.68	01/12/2009	11/12/2009
442	Consultant 4	0	27221	5360360	3710288	6727300	3172609	4663267	1998824	1.44	2.12	2.33	01/12/2009	11/12/2009
443	Consultant 4	-	14448	4258717	2050157	4756187	1771564	3093653	1042063	2.08	2.68	2.97	01/12/2009	11/12/2009
444	Consultant 4	0.3	225	55395	13056	42179	9523	17636	3900	4.24	4.43	4.52	09/11/2009	14/11/2009
445	Consultant 4	0.25	475	163629	49157	157483	37531	79230	19731	3.33	4.20	4.02	09/11/2009	14/11/2009
446	Consultant 4	0.3	276	3.2	1.6	3.5	2	3.2	2	2.00	1.75	1.60	01/04/2010	12/04/2010
447	Consultant 4	-	321	4.8	2.5	5.5	2.5	4	2.1	1.92	2.20	1.90	01/04/2010	12/04/2010
448	Consultant 4	0.3	5825	6.5	2	6.5	2	4.2	1.5	3.25	3.25	2.80	01/04/2010	12/04/2010
449	Consultant 4	0.2	3405	6.8	2.5	7.7	2.4	6	2.2	2.72	3.21	2.73	01/04/2010	12/04/2010
450	Consultant 4	0.3	7286	1251998	233952	1057451	201853	790460	268698	5.35	5.24	2.94	07/01/2010	18/01/2010
451	Consultant 4	0.55	72	1.3	2.3	4.5	3	6.9	2.9	0.57	1.50	2.38	20/06/2010	27/08/2010
452	Consultant 4	0.4	1227	2.5	3	4.2	2.4	3.2	1.5	0.83	1.75	2.13	20/06/2010	25/07/2010
453	Consultant 4	0.5	6315	5.3	1.8	4.2	1	2.3	0.8	2.94	4.20	2.88	13/08/2010	31/08/2010
454	Consultant 4	0.25	11078	5.8	1.3	4	0.7	2.1	0.5	4.46	5.71	4.20	13/08/2010	31/08/2010
455	Consultant 4	0.2	2045	7	1.5	5	0.9	2.8	0.6	4.67	5.56	4.67	13/08/2010	31/08/2010
456	Consultant 4	0.2	4265	5.5	2.5	7	1.9	5.5	1.5	2.20	3.68	3.67	13/08/2010	31/08/2010
457	Consultant 4	-	2860	6.3	1.5	4.8	1	2.5	0.6	4.20	4.80	4.17	13/08/2010	31/08/2010
458	Consultant 4	0.5	730	207682	35783	145899	23864	79302	18990	5.80	6.11	4.18	02/01/2010	22/01/2010

No.	Consultant	Depth	Concentration (mg/kg)	nC_{12}	iC_{14}	nC_{13}	iC_{15}	nC_{14}	iC_{16}	nC_{12} : iC_{14}	nC_{13} : iC_{15}	nC_{14} : iC_{16}	Release Date	Sampling Date
459	Consultant 4	0.2	4308	1088973	202671	793845	131216	437285	105697	5.37	6.05	4.14	02/01/2010	22/01/2010
460	Consultant 4	0.35	288	1.6	2.9	2.5	4	2.2	3.2	0.55	0.63	0.69	01/12/2009	15/10/2010
461	Consultant 4	0.1	3741	5.5	1.4	5.2	1.2	3.8	0.9	3.93	4.33	4.22	01/12/2009	15/10/2010
462	Consultant 4	0.25	510	5.5	1.4	5.5	1	3.4	0.8	3.93	5.50	4.25	01/12/2009	15/10/2010
463	Consultant 4	0.5	149	3.5	1	3.5	0.9	2.6	0.8	3.50	3.89	3.25	01/12/2009	15/10/2010
464	Consultant 4	1	5269	2081830	590337	1696783	275561	706851	148839	3.53	6.16	4.75	10/12/2009	14/12/2009
465	Consultant 4	0.9	112	49660	17387	57445	11061	32452	7206	2.86	5.19	4.50	10/12/2009	14/12/2009
466	Consultant 4	1	2177	759161	210429	524250	104289	252200	53368	3.61	5.03	4.73	10/12/2009	14/12/2009
467	Consultant 4	0.4	4497	1228896	421754	868067	197326	466802	104433	2.91	4.40	4.47	10/12/2009	14/12/2009
468	Consultant 4	0.3	15635	6.9	1.4	5.5	0.8	3.6	0.8	4.93	6.88	4.50	07/10/2010	28/10/2010
469	Consultant 4	0.1	51984	6.5	1.5	7.2	1.5	6.6	1.8	4.33	4.80	3.67	07/10/2010	28/10/2010
470	Consultant 4	0.1	11948	6.2	1.5	6.3	1	4.5	1	4.13	6.30	4.50	07/10/2010	28/10/2010
471	Consultant 4	-	17286	7.7	1.5	6.3	1	4.5	1	5.13	6.30	4.50	07/10/2010	28/10/2010
472	Consultant 4	0.45	4301	7.6	1.6	6.5	1.7	2.8	1.1	4.75	3.82	2.55	23/10/2010	15/11/2010
473	Consultant 4	0.5	5910	777784	287576	594670	226291	287585	122352	2.70	2.63	2.35	21/10/2009	01/12/2009

11.0 Appendix 4 Cross Validation Tables

Table 11.1 Cross Validation - Regression Models

Sample Number	$nC_{14} : iC_{16}$	Release Days	Exponential Model				Linear Model				Power Model				Logarithmic Model				Polynomial Model			
			Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error
18	6.44	1	1	0	0	0	-8	-9	9	73	1	0	0	0	28	27	27	751	4	3	3	6
201	6.55	1	1	0	0	0	-9	-10	10	91	1	0	0	0	28	27	27	741	4	3	3	11
202	6.13	1	1	0	0	0	-5	-6	6	34	2	1	1	1	29	28	28	782	2	1	1	1
242	5.76	2	2	0	0	0	-2	-4	4	14	2	0	0	0	30	28	28	770	1	-1	1	1
243	5.54	2	2	0	0	0	0	-2	2	4	2	0	0	0	30	28	28	796	1	-1	1	1
244	6.18	2	1	-1	1	1	-5	-7	7	54	2	0	0	0	29	27	27	724	2	0	0	0
245	5.21	2	3	1	1	1	3	1	1	0	3	1	1	1	31	29	29	840	1	-1	1	0
193	4.47	3	5	2	2	4	8	5	5	28	5	2	2	2	33	30	30	902	5	2	2	3
344	4.88	3	4	1	1	0	5	2	2	5	3	0	0	0	32	29	29	832	2	-1	1	0
345	4.43	3	5	2	2	5	9	6	6	31	5	2	2	3	33	30	30	910	5	2	2	3
346	4.15	3	7	4	4	14	11	8	8	60	6	3	3	8	34	31	31	967	7	4	4	15
352	4.44	4	5	1	1	1	9	5	5	20	5	1	1	0	33	29	29	849	5	1	1	1
464	4.75	4	4	0	0	0	6	2	2	5	4	0	0	0	32	28	28	796	3	-1	1	1
466	4.73	4	4	0	0	0	6	2	2	5	4	0	0	0	32	28	28	800	3	-1	1	1
467	4.47	4	5	1	1	1	8	4	4	18	5	1	1	0	33	29	29	843	5	1	1	0
50	4.99	5	3	-2	2	3	4	-1	1	1	3	-2	2	4	32	27	27	707	2	-3	3	9

Sample Number	$nC_{14} : iC_{16}$	Release Days	Exponential Model				Linear Model				Power Model				Logarithmic Model				Polynomial Model			
			Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error
51	4.86	5	4	-1	1	2	5	0	0	0	3	-2	2	3	32	27	27	724	2	-3	3	6
338	4.00	5	7	2	2	6	12	7	7	46	6	1	1	2	35	30	30	877	8	3	3	9
339	4.39	5	5	0	0	0	9	4	4	15	5	0	0	0	33	28	28	800	5	0	0	0
435	4.82	5	4	-1	1	2	6	1	1	0	3	-2	2	2	32	27	27	730	3	-2	2	6
437	4.62	5	4	-1	1	0	7	2	2	4	4	-1	1	1	33	28	28	761	4	-1	1	2
38	4.55	6	5	-1	1	2	8	2	2	3	4	-2	2	3	33	27	27	719	4	-2	2	4
39	4.50	6	5	-1	1	1	8	2	2	4	4	-2	2	3	33	27	27	726	4	-2	2	3
35	4.20	9	6	-3	3	8	10	1	1	1	5	-4	4	13	34	25	25	619	6	-3	3	8
36	4.40	9	5	-4	4	14	9	0	0	0	5	-4	4	19	33	24	24	588	5	-4	4	18
438	3.24	10	14	4	4	15	18	8	8	58	13	3	3	6	38	28	28	772	16	6	6	38
439	3.72	10	9	-1	1	0	14	4	4	15	8	-2	2	4	36	26	26	655	11	1	1	0
440	3.29	10	13	3	3	11	17	7	7	52	12	2	2	4	38	28	28	757	15	5	5	30
240	3.01	12	17	5	5	23	19	7	7	55	16	4	4	15	39	27	27	725	19	7	7	53
46	3.03	15	16	1	1	2	19	4	4	17	15	0	0	0	39	24	24	555	19	4	4	15
77	3.62	15	10	-5	5	25	15	0	0	0	9	-6	6	40	36	21	21	433	11	-4	4	12
78	3.30	15	13	-2	2	4	17	2	2	4	12	-3	3	11	37	22	22	491	15	0	0	0
453	2.88	18	19	1	1	0	20	2	2	5	18	0	0	0	39	21	21	449	21	3	3	9
275	2.65	19	22	3	3	12	22	3	3	9	24	5	5	23	41	22	22	464	25	6	6	31
96	2.98	20	17	-3	3	10	19	-1	1	1	16	-4	4	16	38	18	18	339	19	-1	1	1
472	2.55	23	24	1	1	2	23	0	0	0	27	4	4	16	41	18	18	315	26	3	3	10
107	2.76	24	20	-4	4	15	21	-3	3	10	20	-4	4	13	39	15	15	233	22	-2	2	2

Sample Number	$nC_{14} : iC_{16}$	Release Days	Exponential Model				Linear Model				Power Model				Logarithmic Model				Polynomial Model			
			Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error	Predicted	Residual	Absolute Value	Squared Error
256	2.68	24	22	-2	2	6	21	-3	3	7	23	-1	1	2	40	16	16	249	24	0	0	0
387	2.18	29	33	4	4	15	25	-4	4	17	46	17	17	275	43	14	14	187	33	4	4	13
55	2.48	30	25	-5	5	24	23	-7	7	56	29	-1	1	1	40	10	10	105	27	-3	3	11
258	2.65	31	22	-9	9	85	21	-10	10	96	23	-8	8	62	39	8	8	66	24	-7	7	52
452	2.13	35	34	-1	1	2	25	-10	10	105	49	14	14	183	42	7	7	48	33	-2	2	6
473	2.35	40	28	-13	13	180	23	-18	18	334	34	-7	7	51	39	-2	2	3	28	-13	13	176
Mean Squared Error (MSE)			**MSE =**			**12**	**MSE =**			**32**	**MSE =**			**18**	**MSE =**			**614**	**MSE =**			**13**

Table 11.2 Cross Validation – Christensen and Larsen Linear Regression Model.

Site Locations	nC_{17}/Pristane	Age	Predicted	Residual	Abs. Value	Squared Error
Provestenen	0.08	22	18	-4	4	13
Ishoj	0.1	18	19	1	1	1
Fredericia	0.05	18	20	2	2	2
Hengelo, depot	0.31	19	17	-2	2	5
Haarlem	0.52	17	15	-2	2	4
Vanlose	0.26	14	18	4	4	16
Horsholm	0.94	12	12	0	0	0
Nieuwesluis	0.88	11	12	1	1	2
Bruunok	1.08	9	11	2	2	3
Thisted	1.34	8	8	0	0	0
Hengelo, loading rack	1.62	9	5	-4	4	14
Ejby	2.19	0.2	2	2	2	4
				Mean Squared Error (MSE)		5

Table 11.3 Cross Validation – Hurst Linear Regression Model.

nC_{17}/Pristane	Age	Predicted	Residual	Abs. Value	Squared Error
2.12	0	1	1	1	0
2.1	0	1	1	1	1
2	0	2	2	2	4
2.23	0.5	-1	-1	1	1
2.08	0.5	1	0	0	0
1.95	0.5	2	2	2	3
2	4.5	1	-3	3	10
1.8	4	4	0	0	0
1.6	9	5	-4	4	14
1.3	8	8	0	0	0
1.3	8.5	8	0	0	0
1.05	9.5	11	1	1	1
1	10	11	1	1	1
0.85	11	13	2	2	2
0.9	12	12	0	0	0
0.65	14	14	0	0	0
0.6	14.5	15	0	0	0
0.5	15	16	1	1	1
0.3	14	18	4	4	15
0.5	17	16	-1	1	2
0.2	17	19	2	2	3
0.21	17.5	19	1	1	1

nC_{17}/Pristane	Age	Predicted	Residual	Abs. Value	Squared Error
0.25	18	18	0	0	0
0.3	18.5	18	-1	1	1
0.31	19	18	-1	1	2
0.08	18	20	2	2	4
0.1	18.5	20	1	1	1
0.05	19	20	1	1	1
0.1	21	19	-2	2	2
0.25	21	18	-3	3	9
0.15	21.5	19	-3	3	6
0.12	21.5	19	-2	2	5
0	21.5	20	-1	1	1
				Mean Squared Error (MSE)	3

Table 11.4 Exponential Excluding Samples 55, 77, 96, 107, 256, 258, 452 and 473.

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
18	6.44	1	1	0	0	0	1
201	6.55	1	1	0	0	0	1
202	6.13	1	1	0	0	0	1
242	5.76	2	2	0	0	0	2
243	5.54	2	2	0	0	0	2
244	6.18	2	1	-1	1	1	1
245	5.21	2	3	1	1	1	3
193	4.47	3	5	2	2	4	5
344	4.88	3	4	1	1	0	4
345	4.43	3	5	2	2	4	5
346	4.15	3	6	3	3	11	6
352	4.44	4	5	1	1	1	5
464	4.75	4	4	0	0	0	4
466	4.73	4	4	0	0	0	4
467	4.47	4	5	1	1	1	5
50	4.99	5	3	-2	2	3	3
51	4.86	5	4	-1	1	2	4
338	4.00	5	7	2	2	4	7
339	4.39	5	5	0	0	0	5
435	4.82	5	4	-1	1	2	4
437	4.62	5	4	-1	1	0	4
38	4.55	6	5	-1	1	2	5
39	4.50	6	5	-1	1	2	5

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
35	4.20	9	6	-3	3	10	6
36	4.40	9	5	-4	4	16	5
438	3.24	10	12	2	2	5	12
439	3.72	10	8	-2	2	2	9
440	3.29	10	12	2	2	3	12
240	3.01	12	15	3	3	7	14
46	3.03	15	14	-1	1	1	14
78	3.30	15	11	-4	4	13	12
453	2.88	18	16	-2	2	5	16
275	2.65	19	19	0	0	0	19
472	2.55	23	20	-3	3	8	21
387	2.18	29	27	-2	2	6	27
	Maximum			3			
	Minimum			-4			
	Mean Squared Error (MSE)					3	

Table 11.5 Linear Excluding Samples 18, 46, 55, 193, 201, 202, 240, 242, 244, 258, 338, 339, 345, 346, 352, 387, 438, 439, 440, 452, 467 and 473.

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
243	5.54	2	-1	-3	3	10	-1
245	5.21	2	2	0	0	0	2
344	4.88	3	5	2	2	5	6
464	4.75	4	6	2	2	3	4
466	4.73	4	6	2	2	5	5
50	4.99	5	4	-1	1	2	5
51	4.86	5	5	0	0	0	7
435	4.82	5	5	0	0	0	7
437	4.62	5	7	2	2	3	7
38	4.55	6	7	1	1	1	10
39	4.50	6	8	2	2	2	8
35	4.20	9	10	1	1	1	14
36	4.40	9	8	-1	1	1	17
77	3.62	15	14	-1	1	1	20
78	3.30	15	17	2	2	3	22
453	2.88	18	20	2	2	5	19
275	2.65	19	22	3	3	10	22
96	2.98	20	19	-1	1	1	21

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
472	2.55	23	22	-1	1	0	21
107	2.76	24	20	-4	4	14	-1
256	2.68	24	21	-3	3	9	2
	Maximum		3				
	Minimum		-4				
	Mean Squared Error (MSE)					3	

Table 11.6 Power Excluding Samples 77, 240, 258, 275, 387, 452 and 472.

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
18	6.44	1	1	0	0	0	1
201	6.55	1	1	0	0	0	1
202	6.13	1	2	1	1	0	2
242	5.76	2	2	0	0	0	2
243	5.54	2	2	0	0	0	2
244	6.18	2	1	-1	1	0	2
245	5.21	2	3	1	1	1	3
193	4.47	3	5	2	2	2	5
344	4.88	3	3	0	0	0	3
345	4.43	3	5	2	2	3	5
346	4.15	3	6	3	3	8	6
352	4.44	4	5	1	1	0	5
464	4.75	4	4	0	0	0	4
466	4.73	4	4	0	0	0	4
467	4.47	4	5	1	1	0	5
50	4.99	5	3	-2	2	4	3
51	4.86	5	3	-2	2	3	3
338	4.00	5	7	2	2	2	7
339	4.39	5	5	0	0	0	5
435	4.82	5	3	-2	2	2	3
437	4.62	5	4	-1	1	1	4
38	4.55	6	4	-2	2	3	4
39	4.50	6	4	-2	2	3	4
35	4.20	9	5	-4	4	12	6
36	4.40	9	5	-4	4	19	5
438	3.24	10	13	3	3	12	13
439	3.72	10	8	-2	2	3	8
440	3.29	10	13	3	3	7	13

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
46	3.03	15	17	2	2	3	17
78	3.30	15	12	-3	3	7	12
453	2.88	18	20	2	2	4	20
96	2.98	20	17	-3	3	7	17
472	2.55	23	27	4	4	16	26
107	2.76	24	23	-1	1	2	23
256	2.68	24	25	1	1	2	25
55	2.48	30	33	3	3	8	32
473	2.35	40	39	-1	1	2	39
	Maximum			-4			
	Minimum			3			
	Mean Squared Error (MSE)					3	

Table 11.7 Polynomial Excluding Samples 36, 46, 55, 77, 240, 258, 275, 346, 387, 438, 440 and 473.

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
18	6.44	1	2	1	1	2	2
201	6.55	1	3	2	2	4	2
202	6.13	1	1	0	0	0	1
242	5.76	2	1	-1	1	1	1
243	5.54	2	1	-1	1	1	1
244	6.18	2	1	-1	1	0	1
245	5.21	2	2	0	0	0	2
193	4.47	3	5	2	2	5	5
344	4.88	3	3	0	0	0	3
345	4.43	3	5	2	2	6	5
352	4.44	4	5	1	1	2	5
464	4.75	4	4	0	0	0	4
466	4.73	4	4	0	0	0	4
467	4.47	4	5	1	1	1	5
50	4.99	5	2	-3	3	7	3
51	4.86	5	3	-2	2	4	3
338	4.00	5	9	4	4	13	8
339	4.39	5	6	1	1	0	6
435	4.82	5	3	-2	2	3	3
437	4.62	5	4	-1	1	1	4
38	4.55	6	5	-1	1	2	5
39	4.50	6	5	-1	1	1	5

Sample Number	nC14 : iC16	Age	Predicted	Residual	Abs. Value	Squared Error	Final Model Prediction Estimate
35	4.20	9	7	-2	2	5	7
439	3.72	10	11	1	1	1	11
78	3.30	15	15	0	0	0	15
453	2.88	18	21	3	3	9	21
96	2.98	20	19	-1	1	1	19
472	2.55	23	26	3	3	9	25
107	2.76	24	22	-2	2	4	22
256	2.68	24	23	-1	1	0	24
452	2.13	35	31	-4	4	20	32
	Maximum			4			
	Minimum			-4			
	Mean Squared Error (MSE)					**3**	

12.0 References

Acher, A.J., Boderie, P. and Yaron, B. (1989) Soil pollution by petroleum products, I. Multiphase migration of kerosene components in soil columns. **Journal of Contaminant Hydrology** 4: pp.333-345.

Alimi, H. (2002) Invited Commentary on the Christensen and Larsen Technique. **Environmental Forensics** 3, pp.5.

Alimi, H., Ertel, T. and Schug B. (2003) Contributed Articles - Fingerprinting of Hydrocarbon Fuel Contaminants: Literature Review. **Environmental Forensics** pp.25-38.

AnalChem Resources (2005) **Practical Gas Chromatography** [online]. Available at: http://delloyd.50megs.com/whats_new.html#theory [Accessed October 2010].

Anderssen, E., Dyrstad, K., Westad, F. and Martens, H. (2006) Reducting over-optimism in variable selection by cross-model validation. **Chemometrics and Intelligent Laboratory Systems** 85, pp.69-74.

Anonymous (Anon.). (2010a) **Gas Chromatography** [online]. Available at: http://www.ux1.eiu.edu/~cfdgk/chm3780fa08/instrumentconcepts/gc.pdf [Accessed October 2010].

Anonymous (Anon.). (2010b) **Chapter 1 Introduction to Principles and Practice of Mass Spectrometry** [online]. Available at: http://www.rsc.org/ebooks/archive/free/BK9780854045716/BK9780854045716-00001.pdf [Accessed October 2010].

Agency for Toxic Substance and Diease Registry (ASTDR) (1995) **Toxicological Profile for Fuel Oils.** [online]. Available at: http://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=516&tid=91 [Accessed 10 May 2012].

Atlas, R.M. and Bartha, R. (1992) Hydrocarbon Biodegradation and Oil Spill Bioremediation. **Advances in Microbial Ecology** 12, pp.287 - 315.

Agilent (2009) **Understanding Your ChemStation** [online]. Available at: http://www.chem.agilent.com/Library/usermanuals/Public/G2070-91126_Understanding.pdf [Accessed 12th March 2012].

Bergmeir, C. and Benitez, J.M. (2012) On the use of cross-validation for time series predictor evaluation. **Information Sciences 191**, pp.192-213.

Binazadeh, M., Karimi, I.A. and Li, Z. (2009) Fast biodegradation of long chain n-alkanes and crude oil at high concentrations with *Rhodococcus* sp. Moj-3449. **Enzyme and Microbial Technology** 45: pp.195-202.

Brusby, R., Custy, D. and Woolley, D. (2001) **Report on - Oil Contamination – The Role of Environmental Health Following an Accidental Release of Central Heating Oil** [online] . Available at: **http://www.uwe.ac.uk/aqm/centre/region/bqs/bqs_oil_spills.pdf**.

Brilis, G.M. (2002) Invited Commentaries on the Christensen and Larsen Technique. **Environmental Forensics** 3, pp.3.

Bregnard, T. P-A., Hohener, P., Haner, A. and Zeyer, J. (1996) Degradation of weathered diesel fuel by microorganisms from a contaminated aquifer in aerobic and anaerobic microcosms. **Environmental Toxicology and Chemistry** 15: pp.299–307.

Bobra, M. (1992) Solubility Behaviour of Petroleum Oils in Water Ottawa, ON: **Environment Canada**, Environmental Protection Directorate.

Borra, S. and Ciaccio, A.Di. (2010) Measuring the prediction error. A comparison of cross-validation bootstrap and covariance penalty methods. **Computational Statistics and Data Analysis** 54, pp.2976-2989.

Chapelle, F.H. (2001) **Ground-water Microbiology and Geochemistry, 2nd** ed. New York, NY: John Wiley & Sons.

Christensen, L. B. and Larsen, T. H. (1993) Method for Determining the Age of Diesel Oil Spills in the Soil. **Groundwater Monitoring Review** pp.142 - 149.

Chung, C. and Fabbri, A.G. (2008) Predicting landslides for risk analysis – Spatial models tested by a cross-validaque. **Geomorphology** 99: pp.438-452.

Clemson University (CL) (2000) **Linear Regression and Excel – Physics Laboratory Excel Tutorial #11** [online]. Available at: http://phoenix.phys.clemson.edu/tutorials/excel/regression.html [Accessed November 2010].

Costa, H. and Sauer, T.C. (2005) Forensic Approaches and Considerations in Identifying PAH Background. **Environmental Forensics** 6, pp.9-16.

Costa, H., White K.A. and Ruspantini J.J. (2004) Distinguishing PAH Background and MGP Residues in Sediments of a Freshwater Creek. **Environmental Forensics** 5, pp.171-182.

Dashti, N., Al-Awadhi, H., Khanafer, M., Abdelghany, S. and Radwan, S. (2008) Potential of hexadecane-utilizing soil-microorganisms for growth on hexadecanol, hexadecanal and hexadecanoic acid as sole sources of carbon and energy. **Chemosphere** 70; pp.475-579.

Davidson, J.M. and Creek, D.N. (2000) Using the Gasoline Additive MTBE in Forensic Environmental Investigations. **Environmental Forensics 1,** pp.31-36.

Davis, A., Howe, B., Nicholson, A., McCaffery, S. and Hoenke, K.A. (2005) Use of Geochemical Forensics to Determine Release Eras of Petrochemicals to Groundwater, Whitehorse, Yukon. **Environmental Forensics** 6, pp.253-271.

DeCoster, J. (1998) **Introductory Statistics Notes** [online]. Department of Psycology, University of Alabama, 348 Gordon Palmer Hall, Box 870348, Tuscaloosa, AL. Available at: http://www.stat-help.com/intro.pdf [Accessed 24 August 2011].

de Jonge, H., Freijer, J.I., Verstraten, J.M., Westerveld, J. and van der Wielen, F.W.M. (1997) Relation between bioavailability and fuel oil hydrocarbon composition in contaminated soils. **Environmental Science & Technology**. 31: pp.771-775.

Department of Energy and Climate Change (DECC) (2008) Crude Oil and Petroleum Products: Imports by Product 1920 to 2008. In: **Change, Department of Energy and Climate Change.** USA.

Department for Environment Food and Rural Affairs (DEFRA) 2010 **Sulphur Content of Liquid Fuels Directives** [online]. Available at: http://www.defra.gov.uk/environment/quality/air/airquality/eu-int/eu-directives/sulphur/ [Accessed 20 August 2010].

Department of Energy and Climate Change (DECC) 2010 **Oil and Gas – Environmental Standards and Product Quality** [online]. Available at: https://www.og.decc.gov.uk/downstream/environment/index.htm [Accessed 20 August 2010].

Directive 98/70/EC of the European Parliament and of the Council of 13 October 1998 relating to the quality of petrol and diesel fuels and amending Directive 93/12/EEC.

Directive 1999/32/EC of 26 April 1999 relating to a reduction in the sulphur content of certain liquid fuels and amending Directive 93/12/EEC.

Directive 2003/17/EC of the European Parliament and of the Council of 3 March 2003 amending Directive 98/70/EC relating to the quality of petrol and diesel fuels.

Dror, I., Gerstl, Z. and Yaron B. (2001) Temporal Changes in kerosene Content and Composition in Field Soil as a Result of Leaching. **Journal of Contaminant Hydrology** 48: pp. 305-323.

Droge, B. (1999) Asymptotic optimality of full cross-validation for selecting linear regression models. **Statistics & Probability Letters** 44: pp.351-357.

Dror, I., Gestl, Z., Prost, R. and Yaaton, B. (2002) Abiotic behavior of entrapped petroleum products in the subsurface during leaching. **Chemosphere** 49: pp.1375-1388.

Dror, I., Gestl, Z. and Yaron, B. (2001) Temporal changes in kerosene content and composition in field soil as a result of leaching. **Journal of Contaminant Hydrology** 48: pp.305-323.

Engineering Statistics Handbook (ESH) (2011) **4.1.4.1. Linear Least Squares Regression** [online]. Available at: http://www.itl.nist.gov/div898/handbook/pmd/section1/pmd141.htm [Accessed 25 August 2011].

Environment Agency (EA) (2010) **Oil Pollution** [online]. Available at http://www.environment-agency.gov.uk/research/library/position/41233.aspx [Accessed 17 August 2010].

Environment Agency (EA) (2012) **The Environment Agency's Monitoring Certification Scheme (*MCERTS*) - Performance Standard for Laboratories Undertaking Chemical Testing of Soil** Version 4, EA, Operator Self Monitoring Team, PO Box 519, Preston, PR5 8QD www.mcerts.net

Excel (2011) **Perform a Regression Analysis** [online]. http://office.microsoft.com/en-us/excel-help/perform-a-regression-analysis-HA001111963.aspx [Accessed 25 August 2011].

Fine, P., Graber, E.R. and Yaron, B. (1997) Soil interactions with petroleum hydrocarbons: Abiotic processes. **Soil Technology** 10: pp.133-153.

Fingas, M. (1995) Review A literature review of the physical and predictive modeling of oil spill evaporation **Journal of Hazardous Materials** 42 pp. 157-175.

Fisher M.J. and Marshall A.P. (2009) Understanding descriptive statistics. **Australian College of Critical Care** 22: pp. 93-97.

Galin, Ts., Gerstl, Z. and Yaron, B. (1990) Soil Pollution by petroleum products, III. Kerosene stability in soil columns as affected by volatilization. **Journal of Contaminant Hydrology** 5: pp.375-385.

Gidskehaug, L., Anderssen, E. and Alsberg, B.K. (2008) Cross model validation and optimization of bilinear regression models. **Chemometrics and Intelligent Laboratory Systems** 93: pp.1-10.

Good, S.J. (2002) Operation and Methods Development for the Varian Saturn 2100D GC/MS. **Cantaurus.** 10: pp.7-13 McPherson College Division of Science and Technology.

Gouda, M.K., Omar, S.H., Chekroud, Z.A. and Nour Eldin, H.M. (2007) Technical Note – Bioremediation of kerosene I: A case study in liquid media. **Chemosphere** 69: pp.1807-1814.

Greer, C.W., Fortin, N., Roy, R., Whyte, L.G. and Lee, K. (2003) Indigenous Sediment Microbial Activity in Response to Nutrient Enrichment and Plant Growth Following a Controlled oil Spill on a Fresh Water Wetland. **Bioremediation Journal** 7, pp.69 - 80.

Hurst, R.W. and Schmidt, G. W. (2005) Age Significance of heptadecane (nC_{17}) / Pristane (Pr) Ratios in Forensic Investigations of Refined Product and Crude oil Releases. **Environmental Geoscience** 12, pp.177 - 192.

Jarsjo, J., Destouni, G. and Yaron, B. (1994) Retention and volatilization of kerosene: Laboratory experiments on glacial and post-glacial soils. **Journal of Contaminant Hydrology** 17: pp.167-185.

Jones J.F. (2003) Petroleum Hydrocarbon and Polyaromatic hydrocarbons. In: Thompson, C.K. and Nathanail, P.C. (Ed.) **Chemical Analysis of Contaminated Land.** Blackwell Publishing Ltd.

Kaplan, I.R. (2002) Invited Commentary on the Christensen and Larsen Technique. **Environmental Forensics** 3, pp.7.

Kaplan, I.R. (2003) Age Dating of Environmental Organic Residues. **Environmental Forensics** 4, pp.95-141.

Kaplan, I. R. and Galperin, Y. (1996a) How to Recognize a Hydrocarbon Fuel in the Environment and Estimate its Age of Release. In: Bois, T. J. and Luther, B. J. (Ed.) **Groundwater and Soil Contamination: Technical**

Preparation and Litigation Management. New York, Wiley Law Publishers, pp.145 - 199.

Kaplan, I. R. and Galperin, Y. (2008) Zero-Order Kinetics Model for the Christensen-Larsen Method for Fuitive Fuel Age Estimates. **Groundwater Monitoring Review** 28, pp.94-97.

Kaplan, I.R., Galperin, Y., Alimi, H., Lee, R-P. and Lu, S.-T. (1996b) Patterns of Chemical Changes During Environmental Alteration of Hydrocarbon Fuels. **Groundwater Monitoring Review** 16, pp.113-125.

Kaplan, I.R., Galperin, Y., Lu, S.-T. and Lee, R.P. (1997) Forensic Environmental Geochemistry: Differentiation of Fuel-types, their Sources and Release Time. **Org. Geochem** 27, pp.289 - 317.

Kaplan, I.R., Lu, S., Alimi, H.M. and MacMurphey, J. (2001) Fingerprinting of High Boiling Hydrocarbon Fuels, Asphalts and Lubricants. **Environmental Forensics** 2, pp.231-248.

Killops, S.D. and Al-Juboori, M. (1990) Characterisation of the Unresolved Complex Mixture (UCM) in the Gas Chromatograms of Biodegraded Petroleums. **Org. Geochem** 15, pp.147-160.

Marshall, A.G. and Podgers, R. (2004) Petroleomics: The Next Grand Challenge for Chemical Analysis. **Accounts of Chemical Research** 37, pp.53-59.

Marshall, G. and Jonker L. (2010) An introduction to descriptive statistics: A review and practical guide. **Radiography** 16: e1-e7.

Marques de Sa, J.P. (2007) **Applied Statistics using SPSS, Statistica, MATLAB and R**, Second Edition, New York

MathHolt (2011) **Exponential Regression** [online]. http://www.youtube.com/watch?v=U_cO6Mhk964 [Accessed 25 August 2011].

McKean, J.W. and Sievers, G.L. (1987) Coefficients of determination for least absolute deviation analysis. **Statistics & Probability Letters** 5: pp. 49-54.

Michaelsen, J. (1987) Cross-Validation in Statistical Climate Forecast Models. **Journal of Climate and Applied Meteorology**, 26, 1589–1600. http://journals.ametsoc.org/doi/pdf/10.1175/1520-0450%281987%29026%3C1589%3ACVISCF%3E2.0.CO%3B2

Morrison, R.D. (2000) Critical Review of Environmental Forensic Techniques: Part I. **Environmental Forensics** 1, pp.157-173.

Nagel, A. (2010) Personal Phone Communication.

Nagel, A. (2012) Personal Communication, e-mail dated 20th April 2012 at 0:42.

Nes, W. R. and McKean, M.L. (1977) **Biochemistry of Steroids and Other Isoprenoids**. University Park Press, Baltimore.

Northern Ireland Environment Agency (NIEA) (2010) **The Oil Care Campaign** [online]. Available at http://www.ni-environment.gov.uk/water-home/waterpollution/pol_prevent/water-pollution-initiatives.htm [Accessed 17 August 2010].

Osuji, L.C., Idung, I.D. and Ojinnaka, M. (2006) Hydrocarbon Speciation by Fingerprinting Technique and Diagnostic Vanadium/Nickel Ratio of Mgbede-20 Oil-Impacted Site in the Niger Delta Basin of Nigeria. **Environmental Forensics** 7, pp.259-265.

Oudijk, G. (2009) Age Dating Heating-Oil Releases, Part 1. Heating-Oil Composition and Subsurface Weathering. **Environmental Forensics** 10, pp.107-119.

Oudijk, G. (2010) Personal Communication, e-mail dated 18th May 2010 at 13:16.

Pasadakis, N., Gidarakos, E., Kanellopoulou, G. and Spanoudakis N. (2008) Identifing Sources of Oil Spills in a Refinery by Gas Chromatography and Chemometrics: A Case Study. **Environmental Forensics** 9: pp.33-39.

Peters, K.E., Walters, C.C. and Moldowan, J.W. (2005) **The Biomarker Guide: 1. Biomarkers and isotopes in the environment and human history.** Cambridge University Press.

Phenonmenex (2010) **Bringing Innovation to Gas Chromatography** [online]. Available at: http://www.phenomenex.com/cms400min/zebrongc.aspx [Accessed October 2010].

Philp, P.R. (2004) Application of stable isotopes and radioisotopes in environmental forensics. In: Murphy, B.L. and Morrison, R.D. (Ed.) **Introduction to Environmental Forensics.** San Diego Elsevier, pp. 99-136.

Philp, P. (1985) **Methods in Geochemistry and Geophysics, 23 Fossil Fuel Biomarkers Applications and Spectra.** Elsevier: New York.

Rao, C.R. and Wu, Y. (2005) Linear model selection by cross-validation. **Journal of Statistical Planning and Interference** 128: pp.231-240.

Refaeilzadeh, P., Tang, L. and Liu, H. (2008) **Cross-Validation** [online]. Arizona State University http://www.public.asu.edu/~ltang9/papers/ency-cross-validation.pdf [Accessed 10th March 2012].

Rong, Y. (2000) Statistical Methods and Pitfalls in Environmental Data Analysis. **Environmental Forensics** 1: pp.213-220.

Roy, A.K. and Sarangi, N. (Eds.)(2008) **Applied Bioinformatics Statistics and Economics in Fisheries Research** New India Publishing Agency Delhi ISBN 10:80-89422-86-3.

Royal Society of Chemistry (RSC) (2010) **Modern Instrumental Techniques for Schools and Colleges** [online]. Available at: http://www.youtube.com/watch?v=J-wao0O0_qM [Accessed October 2010].

Scottish Environment Protection Agency (SEPA) (2010) **The Water Environment (Oil Storage) (Scotland) Regulations 2006** [online]. Available at:

http://www.sepa.org.uk/water/water_regulation/regimes/pollution_control/oil_storage.aspx [Accessed 17 August 2010].

Shabir, G., Afzal, M., Anwar, F., Tahseen, R. and Khalid Z.M. (2008) Biodegradation of Kerosene in Soil by a Mixed Bacterial Culture Under Different Nutrient Conditions. **International Biodeterioration & Biodegradation** 61: pp. 161-166.

Snape, I., Harvey, P.McA., Ferguson, S.H., Rayner, J.L. and Revill, A.T. (2005) Investigation of evaporation and biodegradation of fuel spills in Antarctica I. A chemical approach using GC-FID. **Chemosphere** 61: pp.1485-1494.

Statutory Instruments 1999 No. 3107 Public Health, England and Wales Public Health, Scotland, Public Health, Northern Ireland. The Motor Fuel (Composition and Content) Regulations 1999.

StatTrek (2011) **AP Statistics Tutorial: Least Squares Linear Regression** [online]. Avilable at: http://stattrek.com/ap-statistics-1/regression.aspx [Accessed 24 August 2011].

Stout, S. A., Uhler, A.D. and McCarthy, K.J. (2005) Middle Distillate Fuel Fingerprinting Using Drimane-based Bicyclic Sesqueterpanes. **Environmental Forensics** 6, pp.241-252.

Stout, S. A., Uhler, A.D., McCarthy, K.J. and Emsbo-Mattingly S.D. (2002a) Invited Commentary on the Christensen and Larsen Technique. **Environmental Forensics** 3, pp.9-11.

Stout, S.A., Uhler, A.D., McCarthy, K.J., and Emsbo-Mattingly, S. (2002b) Chemical Fingerprinting of Hydrocarbons In: Morrison, R. D., and Murphy, R.D., (Ed.) **Introduction to Environmental Forensics**. San Diego, Academic Press, pp.137-260.

Tamhane, A.C., and Dunlop, D.D. (2000) **Statistics and Data Analysis from Elementary to Intermediate,** Prentice-Hall, Inc. NJ

Tissot, B.P., and Welte, D.H. (1984) **Petroleum Formation and Occurrence,** New York: Springer-Verlag.

TPHCWG, (1998) **Total Petroleum Hydrocarbon Criteria Working Group Series Volume 2: Composition of petroleum mixtures** Amherst, MA: Amherst Scientific Publishers.

The University of Arizona (TUOA) 2012, **12 Validating the Regression Model** [online]. Available at: http://www.ltrr.arizona.edu/~dmeko/notes_12.pdf [Accessed 9th March 2012].

Tracey, J. (2008) Rising Costs of Home Heating Oil **IPM BBC**[online]. Available at: http://www.bbc.co.uk/blogs/ipm/2008/04/rising_costs_of_home_heating_o.shtml.

United States Environmental Protection Agency (USEPA) (1992) **Preparation of Soil Sampling Protocols: Sampling Techniques and Strategies** Office of Research and Development Washington DC 20460 EPA/600/R-92/128 July 1992.

University of Nebraska-Lincoln (UNL) (2010) **Undergraduate Instrumentation Center** [online]. Available at: http://www.chem.unl.edu/uic/gc-fid.shtml [Accessed October 2010].

Volkman J.K., Revill A.T. and Murray A.P. (1997) **Chapter 8 - Applications of Biomarkers for Identifying Sources of Natural and Pollutant Hydrocarbons in Aquatic Environments.** American Chemical Society.

Wade, M.J. (2001) Age-dating Diesel Fuel Spills: Using the European Empirical Time-based Model in the U.S.A. **Environmental Forensics** 2: pp.347-358.

Walpole R.E. and Myers, R.H. (1989) **Probability and Statistics for Engineers and Scientists Fourth Edition**. Macmillan Publishing Company NY, pp.358

Wang, Z. (2010) Personal communication, e-mail dated 11th May 2010 at 19:32.

Wang, Z., Fingas, M., Landriault, M., Sigouin, L., Feng, Y. and Mullin J. (1997) Using Systematic and Comparative Analytical Data to Identify the Source of an Unknown Oil on Contaminated Birds. **Journal of Chromatography A** 775: pp.251-265.

Wang, Z., Fingas, M., Blenkinsopp, S., Sergy, S., Landriault, M., Sigouin, L. and Lambert, P. (1998) Study of 25 year old Nipis Oil Spill: Persistence of Oil Residues and Comparisons Between Surface and Subsurface Sediments. **Environmental Science & Technology** 32: pp 2222-2232.

Wang, Z. and Fingas M. (2006a) **Chromatographic Analysis of the Environment - Oil and Petroleum Product Fingerprinting Analysis by Gas Chromatographic Techniques**, Taylor & Francis.

Wang, Z. and Stout, S.A. (2007) **Oil Spill Environmental Forensics - Fingerprinting and Source Identification**, London, Elsevier.

Wang, Z., Stout, S.A. and Fingas, M. (2006b) Forensic Fingerprinting of Biomarkers for Oil Spill Characterization and Source Identification. **Environmental Forensics** 7, pp.105 - 146.

WCF Fuels – North West (2010) **Lower Sulphur Levels in our Kerosene and Gas Oil** [online]. Available at: http://www.wcfnw.co.uk/news/article.asp?id=6 [Accessed 20 August 2010].

Wiens, T.S., Dale, B.C., Boyce, M.S. and Kershaw, G.P. (2008) The way k-fold cross-validation of resource selection functions. **Ecological Modelling** 212 pp.244-255.

Yang, Y. (2007) Consistency of Cross Validation for Comparing Regression Procedures. **The Annals of Statistics** Vol. 35, No. 6, pp. 2450-2473.

Zemo, D. (2009) Use of Parent Polycyclic Aromatic Hydrocarbon (PAH) Proportions to Attribute PAH Sources in Sediments: A Case Study from the Pacific Northwest. **Environmental Forensics** 10, pp.229-239.

Zhu X., Venosa A.D., Suidan M.T. and Lee K. (2001). **Guidelines for the bioremediation of marine shorelines and freshwater wetlands.** US Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Land Remediation and Pollution Control Division, 26 W. Martin Luther King Drive, Cincinnati, OH 45268.

Zobell C.E. (1973). **Microbial degradation of oil: present statue, problems, and perspectives. In Ahearn and Meyers (Eds.), The microbial degradation of oil pollutants.** Publication No. LSU-SG-73-01, Louisiana State University, Baton Rouge, LA, pp 3-16.